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EFFECT OF DIET ON THE RESISTANCE OF THE ALBINO RAT TO BACTERIUM ABORTUS [1]

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INTRODUCTION

The albino rat appears to have been used but little in experiments with *Bacterium abortus*, perhaps on account of its comparatively high resistance to this organism.[2] [3] However, since the nutritive requirements of the rat have been studied extensively, it has seemed desirable to ascertain the extent to which the resistance of this animal to *Bact. abortus* is affected by certain dietary deficiencies.

EXPERIMENTAL DATA

The rats used in these experiments were raised in the bureau laboratories from healthy, vigorous, and prolific stock. Mature or nearly mature rats were employed. Unless otherwise stated, the rats were fed a diet fully adequate for excellent growth and reproduction. This diet was made up as follows:

Adequate diet for rats	Per cent
Yellow corn meal	25
Graham flour	25
Oatmeal	24
Dried beef	15
Dried bakers' yeast	4
Calcium carbonate	4
Cod-liver oil	2
Sodium chloride	1

A single strain of *Bact. abortus* which has been propagated in these laboratories for several years was used in all experiments. This strain is pathogenic for guinea pigs and exhibits typical cultural and agglutinating properties. In general, 4 or 5 day bouillon cultures were used for inoculation and feeding tests, but glycerin-agar cultures were fed in a few experiments.

Rats which had been exposed to *Bact. abortus* were usually killed at the end of from four to five weeks. Six dilutions of the blood serum were used for agglutination tests, viz, 1 to 25, 1 to 50, 1 to 100, 1 to 200, 1 to 500, and 1 to 1,000. The internal organs were carefully examined and small quantities of the spleen were transferred to serum-agar slants and spread evenly over the surface. An effort was made to transfer approximately the same quantity of spleen to each tube. The cultures were incubated from six to eight days at 37.5° C., when the colonies of *Bact. abortus* were counted.

[1] Received for publication June 18, 1929; issued February, 1930.

[2] BURNET, E., and LAGOANÈRE, J. L. DE POUVOIR PATHOGÈNE DU M. MELITENSIS ET DU B. ABORTUS POUR LE RAT ET LA SOURIS. Arch. Inst. Pasteur Tunis 13: 182-191. 1924.

[3] HAGAN, W. A. THE SUSCEPTIBILITY OF MICE AND RATS TO INFECTION WITH BACILLUS ABORTUS. Jour. Exp. Med. 36: 727-733. 1922.

FEEDING EXPERIMENTS WITH BACTERIUM ABORTUS

Experiment 1. Adequate Diet

Eighteen rats which had received the previously described adequate diet from birth were fed pure cultures of *Bact. abortus* on several successive days. Four to five weeks later the rats were killed and examined. *Bact. abortus* was isolated from the spleen of only one rat, but agglutination reactions in dilutions ranging from 1 to 25 to 1 to 100 were yielded by the blood serum of eight rats.

Experiment 2. Diet Low in Vitamin A

Six rats which had been fed a diet low in vitamin A from birth were fed a pure culture of *Bact. abortus* on several successive days. The diet was made up as follows:

Diet low in vitamin A	Per cent
Graham flour	36
Oatmeal	40
Dried beef	15
Dried bakers' yeast	4
Calcium carbonate	4
Sodium chloride	1

Three weeks after being fed the organism the rats were killed and examined. *Bact. abortus* was isolated from the spleens of two rats. The serum from one of these reacted to the agglutination test in a dilution of 1 to 25, and that from another rat reacted in the same dilution.

Experiment 3. Diet Very Poor in Vitamin A

Six rats which had been fed the above-described diet from birth were transferred to a ration very much poorer in vitamin A, as follows:

Diet very poor in vitamin A	Per cent
Casein	24
Dried brewers' yeast	5
Ash mixture	4
Hydrogenated cottonseed oil	10
Cassava starch	57

The casein was purified from vitamin A by heating in a current of air at from 115 to 130° C. The other constituents have been found to be practically free from this vitamin. This ration is probably poor in vitamin D also, but the rats used appear to have had a very small requirement for this vitamin, apparently because of considerable sunlight which entered the room through the partly opened windows.

After this group of rats had received the foregoing diet for 102 days they were fed a pure culture of *Bact. abortus* on five successive days. Five weeks later they were killed and examined. *Bact. abortus* was isolated from the spleen of only one rat, which also yielded an agglutination reaction in a dilution of 1 to 25. Agglutination reactions were obtained with the blood of two other rats in a dilution of 1 to 50. At the end of the test one rat was in very poor condition but the others were in fair condition. None had ophthalmia.

Experiment 4. Adequate Diet

Four rats which had received an adequate diet from birth were fed approximately 10 c. c. of the contents of the stomach of an aborted calf infected with *Bact. abortus*. Five weeks later the rats were killed and examined. *Bact. abortus* was isolated from the spleen of one rat, but no agglutination reactions were obtained.

The results of these four experiments indicate that the albino rat is not readily infected with *Bact. abortus* when fed a pure culture of the organism, whether the rats have had an adequate diet or one deficient in vitamin A. Thus, of 34 rats which were used in these feeding tests only five were found to be infected with *Bact. abortus*. This method of exposing rats to *Bact. abortus*, therefore, was not regarded as satisfactory for testing the effects of deficient diets on the resistance of the rat to this organism.

Inoculation Experiments

Experiment 5. Adequate Diet

Six rats which had been fed an adequate ration were inoculated intra-abdominally with 0.5 c. c. of a bouillon culture of *Bact. abortus* and 37 days later they were killed and examined. *Bact. abortus* was isolated from the spleens of all six rats, the number of colonies on the tubes of serum agar ranging from 1 to 13. All rats afforded a positive agglutination reaction, the titer being 1 to 1,000 or higher. This method of inoculation appeared to be too severe for testing the effects of deficient diets on the resistance of the rat to *Bact. abortus*.

Experiment 6. Adequate Diet

Six rats which had been on an adequate diet were inoculated subcutaneously with 0.5 c. c. each of a bouillon culture of *Bact. abortus*, and 37 days later they were killed and examined. *Bact. abortus* was isolated from the spleens of three rats, the number of colonies per tube ranging from one to five. The agglutination test was positive in all cases in dilutions of 1 to 100 or 1 to 200, the average being 1 to 150.

Experiment 7. Adequate Diet

This experiment was conducted to determine the effects of inoculating rats subcutaneously with *Bact. abortus* as regards (1) the duration of the infection in the spleen, and (2) the persistence of agglutinins in the blood. Forty-six rats which had been fed an adequate ration were inoculated subcutaneously with *Bact. abortus* on the same date, and at intervals thereafter groups of three rats were killed and examined, with the results shown in Table 1.

The data in Table 1 indicate roughly the course of the infection in the spleens of rats inoculated subcutaneously with *Bact. abortus*. As judged by cultures taken from the spleens of rats killed at intervals during this experiment, the rats appeared to harbor large numbers of *Bact. abortus* in their spleens on the sixth day after inoculation, but the infection gradually subsided and had apparently disappeared by the forty-sixth day. The spleens of rats examined on the thirty-fifth and forty-second days, respectively, were only slightly infected.

TABLE 1.—*Duration of Bacterium abortus infection in albino rats following subcutaneous inoculation*

Rat No.	Period following inoculation	Agglutination titer	Colonies in duplicate cultures from spleen [a]		Weight of spleen as percentage of body weight	Rat No.	Period following inoculation	Agglutination titer	Colonies in duplicate cultures from spleen [a]		Weight of spleen as percentage of body weight
			A	B					A	B	
	Days		*Number*	*Number*	*Per cent*		*Days*		*Number*	*Number*	*Per cent*
1	6	1–100	400–500	.....	0.530	24	42	1–1,000	1	0	.529
2	6	1–200	500–600	.....	.627	25	46	1–200	0	0	.518
3	6	1–200	500–600	.....	.725	26	46	1–50	0	0	.436
4	11	1–200	75–100	.....	.475	27	46	1–200	0	0	.629
5	11	1–1,000	75–100	.....	.535	28	56	1–500	0	0	.475
6	11	1–200	100–150	.....	.465	29	56	1–1,000	0	0	.372
7	16	1–50	100–125	75–100	.420	30	56	1–100	0	0	.522
8	16	1–200	2	25	.461	31	65	(b)	0	0	.265
9	16	1–200	75–100	75–100	.483	32	65	1–500	0	0	.324
10	21	1–200	50–75	50–75	.563	33	65	1–200	0	0	.324
11	21	1–100	15–25	15–25	.353	34	80	1–200	0	0	.281
12	21	1–200	100–150	75–100	.508	35	80	1–200	0	0	.298
13	24	1–200	21	15	.433	36	80	1–100	0	0	.275
14	24	1–200	15	6	.508	37	100	(b)	0	0	.244
15	24	1–200	11	15	.296	38	100	1–200	0	0	.305
16	29	1–200	3	0	.502	39	100	1–200	0	0	.327
17	29	1–100	12	9	.486	40	113	1–100	0	0	.297
18	29	1–200	1	1	.569	41	113	(b)	0	0	.252
19	35	1–1,000	0	0	.521	42	113	1–25	0	0	.214
20	35	1–200	0	0	.567	43	122	(b)	0	0	.235
21	35	1–200	1	0	.421	44	122	1–50	0	0	.221
22	42	1–200	0	0	.665	45	122	1–100	0	0	.299
23	42	1–1,000	1	0	.582	46	122	(b)	0	0	.217

[a] In numerous instances the number of colonies of *Bact. abortus* on the surface of agar slants could not be counted accurately. In such cases the number was approximated.
[b] Negative.

There was considerable variation in the agglutination titer of the blood of rats killed at different intervals. Toward the end of the experiment the titer became lower and the blood of some rats did not react.

The relation between the weight of the spleen and the live weight of the rat indicates a material enlargement of that organ from the sixth to the fifty-sixth days, inclusive, after inoculation with *Bact. abortus*, with a gradual reduction in the size of the spleen from that time until the end of the experiment.

The results of this experiment seemed to indicate that subcutaneous inoculation would be a satisfactory method for exposing rats to *Bact. abortus* in order to test their resistance to this organism as affected by diet. Since rats fed an adequate diet were regularly infected with *Bact. abortus* by this method, and since the infection ran a mild course, it seemed probable that adverse effects of deficient diets would become apparent when infection was accomplished by this means. Experiments with rats fed inadequate diets were therefore carried out.

EXPERIMENT 8. DIETS POOR IN VITAMIN A

Twelve rats which had been fed a diet poor in vitamin A[4] since birth were inoculated subcutaneously with *Bact. abortus* and 39 days later they were killed and examined. *Bact. abortus* was isolated from the spleens of 3 of the 12, the average number of colonies

[4] Ration described under experiment 2.

per tube being five. In all rats the agglutination test was positive in dilutions ranging from 1 to 200 to 1 to 1,000, the average being 1 to 567.

A control group of 12 rats which had been fed an adequate diet from birth was inoculated at the same time as the preceding group. Thirty-nine days later the rats were killed and examined. *Bact. abortus* was isolated from the spleens of 6 of the 12, the average number of colonies per tube being five. The serum from all rats reacted to the agglutination test in dilutions ranging from 1 to 25 to 1 to 1,000, the average being 1 to 644.

Ten rats which had been fed a diet low in vitamin A[5] from birth were placed on the following diet, which was very deficient in this vitamin.

Diet very poor in vitamin A	Per cent
Caseine (purified)	24. 3
Bakers' yeast	10. 0
Ash mixture	4. 0
Hydrogenated cottonseed oil	7. 0
Wheat-germ oil	3. 0
Cassava starch	51. 7

The rats were fed this ration for 28 days, when they were inoculated with *Bact. abortus*. After 31 days more on this ration they were killed and examined. *Bact. abortus* was isolated from the spleens of 9 of the 10. The average number of colonies per tube was four. Serum from all rats reacted to the agglutination test in dilutions ranging from 1 to 100 to 1 to 500, the average being 1 to 370. These rats were in fair condition at the end of the test and showed no signs of ophthalmia.

A control group of nine rats which had received an adequate diet from birth was inoculated at the same time as the preceding group. Thirty-two days later these rats were killed and examined. *Bact. abortus* was found in the spleens of 7 of the 9, the average number of colonies per tube being two. All rats afforded a positive agglutination test in dilutions ranging from 1 to 200 to 1 to 1,000, the average being 1 to 567.

The results of these tests indicate no significant difference in the resistance to *Bact. abortus* of rats fed diets deficient in vitamin A as compared with others fed a diet containing an abundance of this vitamin.

Experiment 9. Diets Poor in the Antineuritic Vitamin B

Seven rats which had been fed an adequate diet from birth were placed for 38 days on a ration containing only a small quantity of the antineuritic vitamin B. The rats were then inoculated subcutaneously with *Bact. abortus* and kept on this diet for 32 days longer, when they were killed and examined for the organism. The diet was made up as follows:

Diet low in vitamin B	Per cent
Dried beef	18. 1
Ash mixture	4. 0
Dried bakers' yeast	1. 5
Cod-liver oil	2. 0
Hydrogenated cottonseed oil	8. 0
Cassava starch	66. 4

[5] Ration described under experiment 2.

With the exception of yeast, the constituents of this ration were practically free from vitamin B, the beef having been heated to destroy any of this vitamin normally present. The proportion of yeast in the ration furnished considerably less vitamin B than is required for mature rats, as is indicated by the fact that the rats lost an average of 29 per cent in weight on this diet. After inoculation with *Bact. abortus* the rats were fed this diet for an additional period of 32 days, or a total of 70 days. Most of the animals were in poor condition at the end of this period. *Bact. abortus* was isolated from the spleens of five of the seven rats, the average number of colonies per tube being four. The serum from all rats reacted to the agglutination test in dilutions ranging from 1 to 50 to 1 to 1,000, the average being 1 to 364.

A second group of 10 rats which had previously received an adequate diet was fed for 11 days a ration containing practically no antineuritic vitamin B. This ration was made up as follows:

Diet very poor in vitamin B	Per cent
Dried beef (autoclaved)	19. 1
Ash mixture	4. 0
Cod-liver oil	2. 0
Hydrogenated cottonseed oil	8. 0
Cassava starch	66. 9

The rats were then inoculated subcutaneously with *Bact. abortus* and continued on this diet for 32 days, or a total of 43 days. Two rats died during the test and the others were in very poor condition at the end, having lost 25 per cent in weight. The rats were killed and examined with the result that *Bact. abortus* was isolated from the spleens of seven of the eight rats, the average number of colonies per tube being four. The cultures from the spleen of the other rat were contaminated. Seven of the rats afforded agglutination reactions in dilutions ranging from 1 to 25 to 1 to 1,000, the average being 1 to 339.

A third group of rats which had received an adequate diet from birth was fed the same deficient ration as that fed the preceding group. After 11 days on this diet the rats were inoculated subcutaneously with *Bact. abortus* and were continued on the same diet for 28 days, or a total of 39 days, when they were killed and examined. At this time most of the rats were in very poor condition, having lost an average of 25 per cent in weight during the test. *Bact. abortus* was isolated from the spleens of seven of the eight rats used, the average number of colonies per tube being 30. The serum from all rats reacted to the agglutination test in dilutions ranging from 1 to 50 to 1 to 500, the average being 1 to 331.

As a control, a fourth group of nine rats which had previously received an adequate diet was inoculated subcutaneously with *Bact. abortus* at the same time as Groups 1 and 2 and continued on the same diet for 32 days. The rats were then killed and examined, with the result that *Bact. abortus* was isolated from the spleens of seven of the nine rats, the average number of colonies per tube being two. The serum from all rats reacted to the agglutination test in dilutions ranging from 1 to 200 to 1 to 1,000, the average being 1 to 567.

These tests indicate that a deficiency of the antineuritic vitamin B had no significant effect on the resistance of the rat to *Bact. abortus*. Cultures from the spleens of the third group of rats fed a ration de-

ficient in vitamin B showed considerably more colonies of *Bact. abortus* than cultures from two other groups of rats fed a similar diet, or than cultures from control rats fed an adequate diet. However, this difference may have been due to the fact that the third group of rats was killed on the twenty-eighth day after inoculation, whereas the three other groups were killed on the thirty-second day. In Table 1 it will be seen that the time between inoculation and examination of the rats has an important bearing on the number of colonies of *Bact. abortus* in the spleens. Thus, cultures from rats killed on the twenty-ninth day showed more colonies than cultures from rats killed on the thirty-second day.

Experiment 10. Diet Poor in Vitamin E

Eighteen female rats which had previously received an adequate diet were placed on a diet very deficient in vitamin E for a preliminary period of 105 days. This diet, which has proved to be very inadequate for reproduction, was made up as follows:

Diet poor in vitamin E	Per cent
Casein	24. 3
Ash mixture	4. 0
Dried bakers' yeast	10. 0
Lard	8. 0
Cod-liver oil	2. 0
Cassava starch	51. 7

The rats were then inoculated subcutaneously with *Bact. abortus* and continued on the same diet for 31 days longer, or a total period of 136 days. They were then killed and examined for the organism. *Bact. abortus* was isolated from the spleens of 17 of the 18 rats; cultures from the other rat were contaminated. The average number of colonies per tube was 12. The serum from all rats reacted to the agglutination test in dilutions ranging from 1 to 100 to 1 to 1,000, the average being 1 to 322.

As a control, eight rats which had previously received an adequate diet were inoculated subcutaneously with *Bact. abortus* at the same time as the preceding group. The rats were continued on this diet and 31 days later they were killed and examined, with the following results: *Bact. abortus* was isolated from the spleen of each rat in the control group, the average number of colonies per tube being nine. The serum from each rat reacted to the agglutination test, the dilutions ranging from 1 to 50 to 1 to 500, the average being 1 to 313.

The results of these tests show that the rats fed a diet very poor in vitamin E for 136 days were not materially less resistant to *Bact. abortus* than were control rats fed an adequate diet.

Experiment 11. Diet Poor in Calcium and Phosphorus

Fourteen rats which had previously received an adequate diet were fed one very poor in calcium and phosphorus for 73 days. The diet was made up as follows:

Diet low in calcium and phosphorus	Per cent
Graham flour	94
Dried beef	5
Sodium chloride	1

This food mixture contained 0.37 per cent phosphorus but calcium was not determined. According to Sherman,[6] wheat contains 0.45 per cent calcium, and meat 0.058 gm. calcium per 100 gm. of protein. Since the dried meat in this diet contained 82.7 per cent protein it probably contained approximately 0.048 per cent calcium, or practically the same proportion as was present in the Graham flour. According to McCollum and Simmonds,[7] wheat is too poor in both calcium and phosphorus to meet the needs of the growing rat. The diet above mentioned contained 15 per cent of a mixture of beef and wheat proteins, which is ample for normal growth in rats. The diet was probably poor in vitamin A.

After the rats had been fed the calcium-phosphorus-deficient diet for 73 days they were inoculated subcutaneouly with *Bact. abortus*. The rats were continued on the same diet, and 34 days later, or after a total period of 107 days on this diet, 11 of them were killed and examined for the organism. Five days later the remaining three were killed and examined. The results were as follows: *Bact. abortus* was isolated from the spleens of 7 of the 14, the average number of colonies per tube being eight. The serum from all rats afforded agglutination reactions in dilutions ranging from 1 to 50 to 1 to 1,000, the average being 1 to 525.

As a control, 12 rats, receiving an adequate diet were inoculated subcutaneously with *Bact. abortus* on the same date as the rats fed the calcium-phosphorus-deficient diet. Thirty-nine days later the control rats were killed and examined, with the following results: *Bact. abortus* was isolated from the spleens of 5 of the 12 rats, the average number of colonies per tube being five. All rats afforded agglutination reactions in dilutions ranging from 1 to 25 to 1 to 1,000, the average being 1 to 644.

The results of this experiment indicate that the rats fed a diet poor in calcium and phosphorus for 107 days were not materially less resistant to *Bact. abortus* than were rats fed an adequate diet.

A careful post-mortem examination was performed on all the rats used in these experiments. Definite lesions, such as are commonly found in guinea pigs inoculated with *Bact. abortus*, that is, nodular spleens and livers dotted with necrotic foci, were not observed. Enlargement of the spleen, while not pronounced, seemed to occur frequently in infected rats, as determined by a comparison between the weight of that organ and the live weight of the rat.

SUMMARY OF RESULTS

In this paper are reported the results of feeding experiments with albino rats to determine the effects of certain dietary deficiencies on the resistance of these animals to *Bact. abortus*. Rations deficient in vitamin A, the antineuritic vitamin B, vitamin E, and in calcium and phosphorus, respectively, were without significant effect on the resistance of the rat to this organism.

[6] SHERMAN, H. C. CHEMISTRY OF FOOD AND NUTRITION. Ed. 3, rewritten and enl., p. 590, 593. New York. 1926.

[7] MCCOLLUM, E. V., and SIMMONDS, N. THE NEWER KNOWLEDGE OF NUTRITION; THE USE OF FOODS FOR THE PRESERVATION OF VITALITY AND HEALTH. Ed. 3, entirely rewritten, p. 129. New York. 1925.

TRANSMISSION OF PULLORUM DISEASE (BACILLARY WHITE DIARRHEA) IN INCUBATORS [1] [2] [3]

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HISTORICAL

Since the memorable discovery reported by Rettger [5] in 1900 that a specific white diarrhea of baby chicks was caused by the organism which he isolated and named *Bacterium pullorum* [6] extensive fundamental investigations have been conducted with reference to this disease. With the rapid rise in economic importance of the hatchery industry in this country, however, it is evident that much ground yet remains to be covered in pullorum-disease control. The dissemination of the disease among chicks in incubators is a large factor governing the net profit, of the hatcheryman on the one hand, and the livability and vigor of chicks delivered to the poultryman on the other. Indeed, the problem goes further than this, since it also affects to a large extent the perpetuation of the disease from generation to generation.

In 1926 Hinshaw, Upp, and Moore [7] and Bushnell, Hinshaw, and Payne [8] reported on the transmission of the disease in incubators. These investigators exposed chicks in an incubator to artificial infection consisting of chick down which had been saturated with *Salmonella pullorum* cultures and dried. The chick mortality from pullorum disease in the infected compartment of the incubator was 59.6 per cent, while in a noninfected compartment exposed to the infected compartment mortality was 24.17 per cent. The controls showed no mortality from this cause.

In 1927 Hinshaw [9] called attention to the need of further investigation of the subject and suggested that the following experimental factors be given attention:

(1) The use of various types of incubators.
(2) Dissemination of the disease by naturally infected chicks from eggs of hens reacting to the agglutination test for pullorum disease.

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[1] Received for publication July 6, 1929; issued February, 1930.

[2] The term "bacillary white diarrhea" and its abbreviation "b. w. d.," have acquired considerable standing through long use in the poultry industry, as well as in contemporary research. It has been found convenient to employ the latter designation in portions of this paper. For further information see page 210.

[3] At the suggestion of the Pennsylvania State Department of Agriculture, Rettger in 1928 proposed to substitute the name "pullorum disease" to denote the infection in either young or adult stock. It is so used in this text. See the following publication: RETTGER, L. F., THE NEED OF ACCEPTED SCIENTIFIC STANDARDS AND RIGID ADHERENCE TO THEM IN PULLORUM DISEASE CONTROL. Jour. Amer. Vet. Med. Assoc. (n. s. 27) 74: 453–461. 1929.

[4] This paper reports work conducted under the direction of the committee on b. w. d. research, of the Bureau of Animal Industry, consisting of M. Dorset, chief, Biochemic Division, chairman; M. A. Jull, senior poultry husbandman, Animal Husbandry Division; and Hubert Bunyea, associate veterinarian, Pathological Division.

[5] RETTGER, L. F. SEPTICÆMIA AMONG YOUNG CHICKENS. N. Y. Med. Jour. 71: 803–805, illus. 1900.

[6] In conformity with the latest classification, the organism is now commonly designated as *Salmonella pullorum*.

[7] HINSHAW, W. R., UPP, C. W., and MOORE, J. M. STUDIES IN TRANSMISSION OF BACILLARY WHITE DIARRHEA IN INCUBATORS. Jour. Amer. Vet. Med. Assoc. (n. s. 21) 68: 631–641. 1926.

[8] BUSHNELL, L. D., HINSHAW, W. R., and PAYNE, L. F. BACILLARY WHITE DIARRHEA IN FOWL. Kans. Agr. Expt. Sta. Tech. Bul. 21: 46–49. 1926.

[9] HINSHAW, W. R. THE INCUBATOR AS A MEANS OF TRANSMITTING BACILLARY WHITE DIARRHŒA. World's Poultry Cong. Proc. (1927) 3: 372–374. [1928]

(3) The use, as controls, of pullorum-disease-free chicks, from hens which have passed the agglutination test for the disease.

(4) Bacteriological examination of dead chicks and embryos to determine the presence or absence of pullorum disease.

(5) Maintenance of experimental chicks under isolation for 14 days for observation and determination of pullorum infection.

In 1928 Hinshaw, Scott, and Payne[10] reported on further studies in dissemination of pullorum disease in incubators. In each of eight hatches completed in a forced-draught incubator, they placed a tray of eggs from infected hens in one end of the machine, and a tray of normal eggs adjacent thereto. A third tray of normal eggs, placed in the opposite end of the same incubator, and a fourth tray of normal eggs in a separate machine were used as controls. The average mortality from pullorum disease in the several trays was as follows:

Chicks from eggs of reacting hens	per cent	30. 01
Chicks from adjacent tray (normal exposed)	do	36. 75
Chicks from opposite tray (normal exposed)	do	18. 59
Chicks from control incubator (unexposed)	do	0. 0

FORMULATION OF PROJECT

In recognition of the paramount importance of control of pullorum disease to the poultry industry, the National Poultry Council through Roy E. Waite, of the University of Maryland, in 1927 entered into a cooperative agreement with the Bureau of Animal Industry to furnish funds to inaugurate a study of the disease from various angles of practical interest to the industry.

A committee on b. w. d. research, consisting of M. Dorset, chairman, M. A. Jull, and Hubert Bunyea, was appointed by John R. Mohler, chief of the bureau, to act in conjunction with a committee from the National Poultry Council to perfect the plans for the investigation and to direct the course of its execution.

The opinion of the joint committee was that the most urgent problem to be investigated was the transmission of pullorum disease in various types of incubators. It was agreed that for this investigation it would be necessary to provide two kinds of eggs for hatching—(1) eggs from hens known to be free from pullorum infection and (2) eggs from hens known to harbor that infection.

Arrangements were made by E. W. Sheets, chief of the animal husbandry division, to supply disease-free eggs from Rhode Island Red hens at the United States Animal Husbandry Experiment Farm, Beltsville, Md. These hens had been tested once for pullorum disease by the agglutination method, and all reactors had been carefully removed from the flock.

For a dependable supply of pullorum-infected eggs, it was decided to purchase a number of reacting hens from various sources and to maintain them at the bureau's experiment station at Bethesda, Md. About 175 hens, including Rhode Island Reds, Barred Plymouth Rocks, and a few birds of other heavy breeds were obtained for this purpose, through the cooperation of E. M. Pickens, of the College of Agriculture, University of Maryland, and William Moore, State veterinarian, Raleigh, N. C. These birds were retested upon their arrival at the experiment station, and only the strong reactors were

[10] HINSHAW, W. R., SCOTT, H. M., and PAYNE, L. F. FURTHER STUDIES ON DISSEMINATION OF SALMONELLA PULLORUM INFECTION IN INCUBATORS. Jour. Amer. Vet. Med. Assoc. (n. s. 25) 72: 550–610, illus. 1928.

retained for the use of the experiment. The flock thus assembled was divided into suitable units for breeding, given proper quarters, and mated with vigorous young male birds.

EXPERIMENTAL BUILDINGS AND EQUIPMENT

Through the cooperation of the late E. C. Schroeder, former superintendent of the experiment station, and that of his successor, W. E. Cotton, ample facilities in the form of buildings and suitably fenced grounds were made available at the station for the project. Additional buildings at the station, as well as various kinds of laboratory

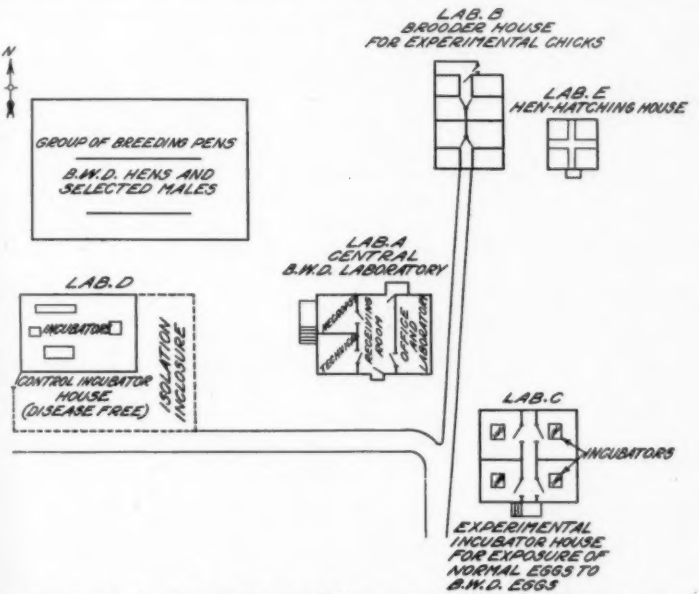

FIGURE 1.—Location and ground plans of buildings used for pullorum-disease research at the experiment station, Bethesda, Md.

and office equipment, were also lent to the project through the cooperation of John S. Buckley, chief of the pathological division. (Fig. 1.)

The buildings occupied by this experiment (fig. 2) consisted of laboratories A, B, C, D, and E, and nine poultry houses and yards. Laboratory A contained the office and bacteriological and pathological laboratory. Laboratory B was the experimental brooder house. Laboratories C and D were the incubator houses, and laboratory E was used for experiments in which hens were used for hatching. Laboratory E was used also for miscellaneous purposes.

Laboratory C was a concrete structure one story high. The floor space was divided into a central hallway running from the front to the back, and four rooms (two on each side), provided with tight, painted walls and seamless waterproof floors, to permit of absolute cleanliness and efficient disinfection of the rooms and their contents.

Each room and the hall were equipped with running water and electricity. The building was uniformly heated by steam heat. There were no communicating doors between rooms. Laboratory C housed the incubators in which the b. w. d. transmission experiments were conducted.

Four different makes of egg incubators were placed in this building, one in each room. These machines will hereinafter be designated

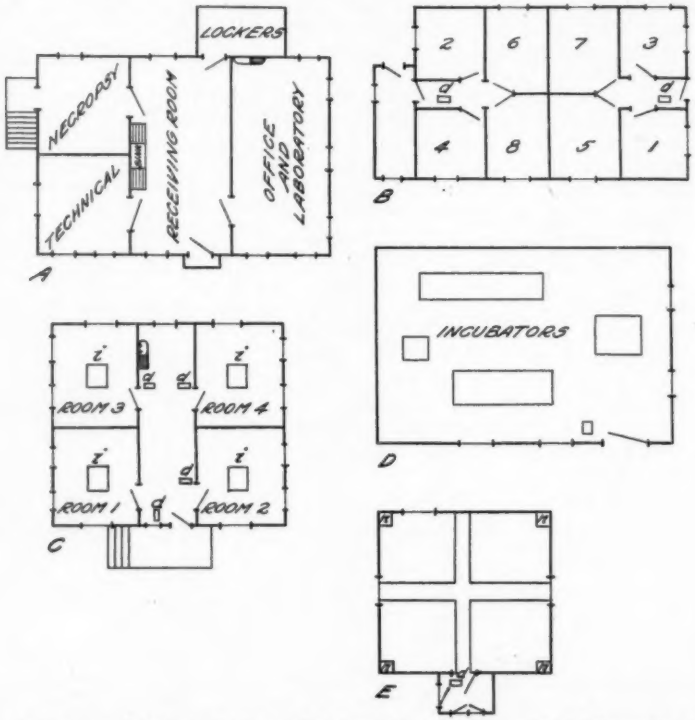

FIGURE 2.—Detailed ground plans of principal experimental buildings used for pullorum-disease research at the experiment station, Bethesda, Md.: *A*, Laboratory A, containing central b. w. d. office and laboratory of pathology and bacteriology; *B*, laboratory B, brooder house for experimental chicks, containing eight isolated brooder rooms with disinfectant pans (*d*); *C*, laboratory C, experimental incubator house for exposure of normal b. w. d. eggs with incubator (*i*) in each room and disinfectant pans (*d*) at doors; *D*, laboratory D, control incubator house for incubation of normal eggs under disease-free conditions; *E*, laboratory E, containing four isolated brooding pens for hen hatching and brooding experiments with nests (*n*) and disinfectant pan (*d*)

respectively as incubators 1, 2, 3, and 4. Incubators 1 and 4 were of the agitated-air type, while 2 and 3 were of the still-air type.

Laboratory D was a frame building about 20 by 30 feet, metal sealed inside, with concrete floor. The building was equipped with electricity and running water. In the center of the ceiling was a trapdoor about 3 by 5 feet opening into the attic. A large ventilator in the roof directly over this trapdoor furnished ventilation for the building.

This building was located about 200 feet from laboratory C, and housed the incubators used for hatching the control eggs. In this building also there were four incubators, one of each of the makes in laboratory C. The incubators in laboratory D were used as control incubators to hatch a portion of the disease-free eggs, in order to establish more fully their freedom from pullorum infection.

Before beginning a hatch the floor of the incubator room was disinfected with a 5 per cent saponified cresol solution. The room was then fumigated with formaldehyde gas, generated by mixing 16⅔ ounces of potassium permanganate with 20 ounces of formalin per 1,000 cubic feet of air space. All openings were closed, the doors being calked with cotton. The rooms thus treated were left closed for a period of more than 18 hours.

The brooder house was a single-story, frame building, 18 by 24 feet, plastered inside and divided across the middle by a solid partition of heavy wall board. Each half of the building was further divided into four rooms of approximately equal proportions, each having a door opening into a common hallway. All joints in the partitions were packed with felt to prevent passage of germ-laden dust from one room to another. Each room had one screened window (except room 3, which had two windows) fitted with a ventilator draft board at the bottom, and a dark, adjustable window shade. An electric outlet and a hot-water radiator were supplied for each room. The doors swung in and were hung on spring hinges to close automatically. They were accurately fitted so as practically to exclude all air, except what was allowed to permeate through the muslin panel in the upper half of the doors.

The rooms at one end of the building were used to house the normal chicks and those in the opposite end to house the b. w. d. chicks. As there was no communication between these two halves of the building, it was necessary to go out around the building to get from one section to the other. This was a sanitary precaution to prevent the transmission of infection from one part of the building to the other. For the same purpose the attendant wore rubber gloves, sleeves, apron, and boots which were disinfected upon entering the building and before entering each brooder room. Visitors were strictly excluded from these rooms during the experiments.

The brooder equipment (fig. 3) for each room was mounted on a painted wooden table and consisted of an electric brooder resting on a ⅜-inch-mesh wire floor within a brooder pan 3 by 5 feet and 3 inches high. The wire cloth floor was 1 inch above the bottom of the brooder pan to allow the droppings of the chicks to fall through beyond their reach. On the sides and one end of the brooder pan were vertical, circular perforations, 1 inch in diameter, outside of which food and water receptacles were placed so as to be constantly accessible to and at the same time prevent contamination of the food and water by the chicks. A 12-inch wire inclosure surmounted the pan on all sides.

Before introducing a brood of chicks into any of these brooders, the room was thoroughly disinfected by spraying with a 5 per cent solution of saponified cresol, and all the equipment in the room was either sprayed or dipped in a like solution.

DEFINITION OF TERMS

It is necessary to explain the meanings of certain abbreviations that appear later in tables and diagrams:

B. w. d. (bacillary white diarrhea) designates fowls that are infected with bacillary white diarrhea (pullorum-disease) infection, or the eggs or chicks from such fowls.

N. e. (normal exposed) designates the eggs or chicks from normal hens (hens free from pullorum disease), such eggs or chicks having been exposed in an incubator to the presence of b. w. d. eggs or chicks.

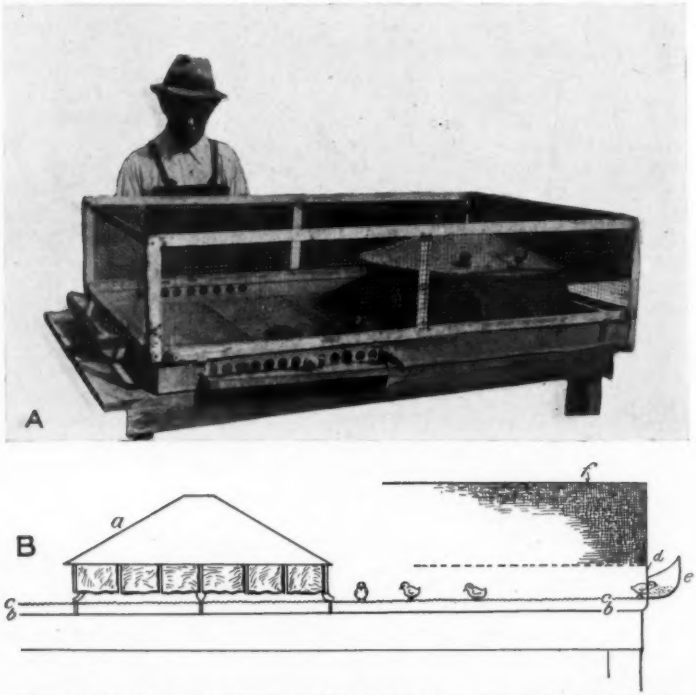

FIGURE 3.—Special brooding equipment used for pullorum-disease experiments: *a*, electric canopy brooder; *b b*, bottom of brooder pan; *c c*, wire-cloth floor; *d*, side of brooder pan perforated; *e*, arrangement for supplying food and water from outside brooder; *f*, 12-inch wire-cloth fence around brooder

N. c. (normal control) designates the eggs or chicks from the same normal flock as the n. e. eggs or chicks, but which have not been exposed to the presence of b. w. d. eggs or chicks.

INCUBATION EXPERIMENTS

Incubators in laboratory C were set with normal and with b. w. d. eggs as is shown in Table 1.

TABLE 1.—*Positions of infected and noninfected eggs in incubators*

Date set	Hatch	Incubator	Positions of infected and noninfected eggs in hatching trays
1928			
Feb. 17	A	1	At opposite ends (cabinet-type incubator).
Feb. 27	A	2	In adjoining compartments (sectional-type incubator).
Feb. 17	A	3	In same compartment; tray partitioned by mesh wire.
Do	A	4	N. e. eggs in upper tray directly over b. w. d. eggs.
Mar. 20	B	1	At opposite ends, same as hatch A 1.
Feb. 28	B	2	In adjoining compartments, same as hatch A 2.
Apr. 5	B	3	Same as hatch A 3.
Mar. 13	B	4	Same as hatch A 4.
Apr. 16	C	1	At opposite ends, same as hatch A 1.
Apr. 23	C	2	In adjoining compartments, same as hatch A 2.
Apr. 30	C	3	Same as hatch A 3.
May 7	C	4	Same as hatch A 4.
May 14	D	1	B. w. d. eggs in upper tray directly over n. e. eggs.
May 22	D	2	In adjoining compartments, same as hatch A 2.
May 28	D	3	Same as hatch A 3.
June 4	D	4	Same as hatch A 4.
June 11	E	1	Same as hatch D 1.
June 18	E	2	In adjoining compartments, same as hatch A 2.
June 25	E	3	Same as hatch A 3.
July 2	E	4	B. w. d. eggs in upper tray directly over n. e. eggs.
July 13	F	1	Same as hatch D.
Aug. 6	G	4	Do.
Aug. 20	H	4	Same as hatch E.
Sept. 14	I	4	Do.
Oct. 1	K	1	Same as hatch D.
Oct. 23	L	4	Same as hatch E.

Some of the b. w. d. eggs used for hatch A in incubators Nos. 1, 3, and 4 were as much as 2 weeks old, as the flock was just coming into production, and the supply of eggs was temporarily limited. None of the eggs set in incubator No. 2 were more than 6 days old.

The eggs in all machines were turned at regular intervals each day, and temperatures and moisture were regulated according to the instructions accompanying each machine.

The room temperatures and incubator temperatures were recorded at least twice each day. These records are available but have not been made a part of this report.

The method of procedure here outlined and the detailed description of the equipment will not be repeated for each experimental hatch, as they were essentially alike for all hatches unless otherwise specifically stated.

BROODING OF CHICKS

After the close of the 21-day period the newly hatched chicks were left in the hatching trays for about 24 hours and were banded with colored celluloid bands for subsequent identification. They were then placed in new, clean chick boxes and taken to their respective brooder rooms in laboratory B, as follows:

Rooms 1, 3, 5, and 7, in the south end of the brooder house, were used to quarter the b. w. d. chicks from the various incubators. Rooms 2 and 4 were used to house the n. e. chicks from the different incubators, while rooms 6 and 8 were used exclusively for the n. c. chicks from the different machines. (Fig. 2, B.) Of course only one brood at a time was allowed in a room, and all rooms were thoroughly disinfected between broods.

After they were 48 hours old, and for the remainder of the 14-day experimental period, the chicks were fed exclusively on a starting mash composed of the following ingredients: Corn meal, fine oatmeal, flour middlings, mineral supplement, meat and bone meal, flake buttermilk, and cod-liver meal. This feed was guaranteed to

contain 14 per cent protein, 4 per cent fat, 3 per cent fiber, and 58 per cent carbohydrates.

The chicks were kept under observation for two weeks. The temperatures of the brooders and rooms were carefully regulated and noted at regular intervals. These records are available but are not included in this report.

All chicks that died within two weeks were autopsied, and cultures made from the liver, lung, and unabsorbed yolk. Any organisms recovered in this way were subjected to further study and tested on various culture media for the characteristics of *Salmonella pullorum*. The following criteria were regarded as sufficient to establish the identity of that organism: Origin, chicken; Gram staining, negative; motility, negative; reaction on litmus milk, slightly acid at 3 days, possibly alkaline in 12 days.

Carbohydrate reactions: Dextrose, acid, with or without gas; lactose, no acid, no gas; saccharose, no acid, no gas; and dulcite, no acid.

A number of representative cultures of *Salmonella pullorum* recovered from each hatch were also tested in beef-extract broths containing 1 per cent, respectively, of maltose, mannite, and levulose, and were examined for motility and Gram-staining qualities. The cultures were also tested by the agglutination method against hyper-immune *S. pullorum* antiserum.

TABLE 2.—*Results of normal control hatches of noninfected eggs, Beltsville, Md.*[1]

Incubator	Eggs set	Fertile eggs	Dead embryos and dead in shell	Chicks hatched
	Number	*Number*	*Number*	*Number*
No. 1	96	74	16	58
No. 3	96	74	14	60
No. 4	96	79	6	73

[1] Owing to the distance between the two experiment stations—Bethesda and Beltsville—and lack of regular transportation facilities, the dead chicks which were sent to the former from the latter place were too far decomposed for bacteriological study when received.

Table 2 gives the results of control hatches in incubators of similar types operated at the United States Animal Husbandry Experiment Farm, about 23 miles from the station at Bethesda. The eggs used in the incubators at Beltsville were of the same age and origin as the normal exposed eggs incubated at Bethesda.

After two hatches had been run in each machine, the control incubators, Nos. 1, 3, and 4, were brought from the Beltsville farm and installed in laboratory D at the Bethesda station. A control incubator of the same make and model as No. 2 was subsequently placed in laboratory D. The new arrangement removed several objectionable features of the former arrangement. As the normal experimental eggs for both the test and the control incubators were taken by automobile from Beltsville to Bethesda any inequality of hatchability otherwise attributable to damage in transit was removed. The chicks that died after being hatched in the control incubators were available for autopsy and bacteriological study while in a fresh condition. In this way the valuable information which otherwise would be lost was conserved.

TABLE 3.—*Results of hatch A*

INCUBATOR NO. 1

Eggs, kind	Eggs set	Fertile eggs	Dead embryos and dead in shell		Chicks					
					Hatched		Died		Dead from which Salmonella pullorum was recovered	
	Number	*Number*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
N. e.	240	198	97	49	101	51	42	42	35	83
B. w. d.	192	173	66	38	107	62	78	73	65	83

INCUBATOR NO. 2

N. e.	150	139	22	16	117	84	16	14	6	38
B. w. d.	144	123	45	37	78	63	58	74	54	93

INCUBATOR NO. 3

N. e.	72	61	39	64	22	36	7	32	6	86
B. w. d.	72	58	31	53	27	47	17	63	12	71

INCUBATOR NO. 4

N. e.	280	245	115	47	130	53	32	25	14	44
B. w. d.	196	172	54	31	118	69	44	37	30	68

TABLE 4.—*Results of hatch B*

INCUBATOR NO. 1

Eggs, kind	Eggs set	Fertile eggs	Dead embryos and dead in shell		Chicks					
					Hatched		Died		Dead from which Salmonella pullorum was recovered	
	Number	*Number*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
N. e.	192	173	29	17	144	83	76	53	70	92
B. w. d.	192	151	38	25	113	75	90	80	86	96

INCUBATOR NO. 2

N. e.	83	62	41	66	21	34	15	71	6	40
B. w. d.	83	67	38	57	29	43	21	72	20	95

INCUBATOR NO. 3

N. e.	72	60	16	27	44	73	19	43	17	89
B. w. d.	72	60	18	30	42	70	26	62	23	88
N. e.	72	60	30	50	30	50	4	13	0	0

INCUBATOR NO. 4

N. e.	168	154	63	41	91	59	18	20	15	83
B. w. d.	168	143	62	43	81	57	30	37	29	97

TABLE 5.—*Results of hatch C*

INCUBATOR NO. 1

Eggs, kind	Eggs set	Fertile eggs	Dead embryos and dead in shell		Chicks					
					Hatched		Died		Dead from which Salmonella pullorum was recovered	
	Number	*Number*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
N. e.	96	78	10	13	68	87	8	12	5	63
B. w. d.	96	75	21	28	54	72	37	69	33	89
N. c.	96	78	16	21	62	79	3	5	0	0
INCUBATOR NO. 2										
N. e.	84	64	19	30	45	70	2	4	0	0
B. w. d.	84	62	23	37	39	63	27	69	24	89
INCUBATOR NO. 3										
N. e.	72	60	18	30	42	70	3	7	1	33
B. w. d.	72	63	28	44	35	56	17	49	16	94
N. c.	72	64	18	28	46	72	0	0	0	0
INCUBATOR NO. 4										
N. e.	96	78	14	18	64	82	2	3	0	0
B. w. d.	96	77	23	30	54	70	6	11	4	67
N. c.	96	86	13	15	73	85	2	3	0	0

TABLE 6.—*Results of hatch D*

INCUBATOR NO. 1

Eggs, kind	Eggs set	Fertile eggs	Dead embryos and dead in shell		Chicks					
					Hatched		Died		Dead from which Salmonella pullorum was recovered	
	Number	*Number*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
N. e.	96	87	17	20	70	80	18	26	16	89
B. w. d.	96	76	23	30	53	70	40	75	36	90
N. c.	96	84	22	26	62	74	5	8	0	0
INCUBATOR NO. 2										
N. e.	84	72	25	35	47	65	3	6	0	0
B. w. d.	84	68	18	26	50	74	30	60	27	90
N. c.	84	62	16	26	46	74	7	15	0	0
INCUBATOR NO. 3										
N. e.	72	62	23	37	39	63	0	0	0	0
B. w. d.	72	62	39	63	23	37	11	48	6	55
N. c.	72	62	16	26	46	74	5	11	0	0
INCUBATOR NO. 4										
N. e.	96	76	20	26	56	74	2	4	0	0
B. w. d.	96	66	23	35	43	65	10	23	8	80
N. c.	96	82	27	33	55	67	4	7	0	0

TABLE 7.—*Results of hatch E*

Eggs, kind	Eggs set	Fertile eggs	Dead embryos and dead in shell		Chicks					
					Hatched		Died		Dead from which Salmonella pullorum was recovered	
	Number	*Number*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cen*
INCUBATOR NO. 1										
N. e.	72	57	7	12	50	88	18	36	16	89
B. w. d.	72	52	17	33	35	67	26	74	26	100
N. e.	66	52	14	27	38	73	4	11	0	0
INCUBATOR NO. 2										
N. e.	72	64	25	39	39	61	4	10	0	0
B. w. d.	72	52	33	63	19	37	10	53	9	90
N. e.	62	51	13	25	38	75	3	8	0	0
INCUBATOR NO. 3										
N. e.	72	57	35	61	22	39	7	32	1	14
B. w. d.	72	50	40	80	10	20	5	50	5	100
N. e.	56	44	15	34	29	66	7	24	0	0
INCUBATOR NO. 4										
N. e.	72	53	24	45	29	55	2	7	2	100
B. w. d.	72	56	22	39	34	61	14	41	9	64
N. e.	69	44	25	57	19	43	2	11	0	0

Tables 3 to 7 constitute a record of all incubation experiments conducted at the Bethesda station in connection with this investigation.

In order to make a comparative test of the dissemination of pullorum disease in hen-hatched chicks a series of hatches was begun at this juncture to develop simultaneously with certain incubator hatches. Each group of settings allotted to hens contained two or three mixed settings and one normal setting. The mixed settings each consisted of 8 b. w. d. eggs and 8 n. e. eggs.

The building used for these tests (fig. 2, E) was of one room with a single doorway opening into an exterior vestibule. The room contained four brooder pens separated from one another by 16-inch alleys. The inclosures were boarded up 1 foot from the floor, and above that poultry wire was stretched to a sufficient height to prevent the birds from leaving their own inclosures. The hatching nests were located in the extreme corners of the room, one in each inclosure. This building and its equipment were thoroughly disinfected before each series of hatches. Tables 8 and 9 show the result of these experiments.

TABLE 8.—*Results of incubator hatches of n. e., b. w. d., and n. c. eggs, to be compared with results of hen-hatched eggs recorded in Table 9*

N. E.

Hatch	Eggs		Dead embryos and dead in shell		Chicks					
	Set	Fertile			Hatched		Died		Dead from which Salmonella pullorum was recovered	
	Number	*Number*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
F	48	34	2	6	32	94	3	9	1	33
G	72	59	18	31	41	69	13	32	11	85
H	72	54	7	13	47	87	4	9	0	0
I	[1] 72									
K	72	55	8	15	47	85	9	19	5	56
L	72	52	11	21	41	79	1	2	0	0
Total or average	408	254	46	18	208	82	30	14	17	57
B. W. D.										
F	72	38	12	32	26	68	17	65	15	88
G	72	60	20	33	40	67	12	30	11	92
H	72	55	19	35	36	65	13	36	11	85
I	[1] 72									
K	72	57	8	14	49	86	10	20	7	70
L	72	55	8	15	47	85	2	4	0	0
Total or average	432	265	67	25	198	75	54	27	44	81
N. C.										
F	120	80	14	16	75	84	2	3	0	0
G	70	53	20	38	33	62	1	3	0	0
H	64	50	5	10	45	90	2	4	0	0
I	108	87	24	28	63	72	0	0	0	0
K	120	87	25	29	62	71	4	6	0	0
L	155	119	29	24	90	76	11	12	0	0
Total or average	637	485	117	24	368	76	20	5	0	0

[1] None hatched.

TABLE 9.—*Results of hen hatches of n. e., b. w. d., and n. c. eggs, to be compared with results of incubator-hatched eggs recorded in Table 8*

N. E.

Hatch	Eggs			Dead embryos and dead in shell [1]		Chicks					
	Set	Fertile	Broken			Hatched [1]		Died		Dead from which Salmonella pullorum was recovered	
	No.	*No.*	*No.*	*No.*	*Per ct.*	*No.*	*Per ct.*	*No.*	*Per ct.*	*No.*	*Per ct.*
F	24	22	6	3	19	13	81	1	8	1	100
G	24	24	4	5	25	15	75	2	13	1	50
H	24	22	3	7	37	12	63	0	0	0	0
I	24	20	1	6	32	13	68	1	8	1	100
K	24	21	5	4	25	12	75	3	25	1	33
Total or average	120	109	19	25	28	65	72	7	11	4	57

[1] Hatchability is figured on number of eggs set less broken eggs and infertile eggs.

TABLE 9.—*Results of hen hatches of n. e., b. w. d., and n. c. eggs, to be compared with results of incubator-hatched eggs recorded in Table 8*—Continued

B. W. D.

Hatch	Eggs			Dead embryos and dead in shell		Chicks					
	Set	Fertile	Broken			Hatched		Died		Dead from which Salmonella pullorum was recovered	
	No.	*No.*	*No.*	*No.*	*Per ct.*	*No.*	*Per ct.*	*No.*	*Per ct*	*No.*	*Per ct.*
F	24	18	2	7	44	9	56	2	22	2	100
G	24	21	3	6	33	12	67	0	0	0	0
H	24	20	5	5	33	10	67	2	20	1	50
I	24	21	3	9	50	9	50	0	0	0	0
K	24	21	6	5	33	10	67	0	0	0	0
Total or average	120	101	19	32	39	50	61	4	8	3	75

N. C.

F	16	16	2	5	36	9	64	0	0	0	0
G	16	16	5	4	36	7	64	0	0	0	0
H	16	12	2	1	10	9	90	0	0	0	0
I	16	12	0	1	8	11	92	0	0	0	0
K	16	16	9	4	57	3	43	3	100	0	0
Total or average	80	72	18	15	28	39	72	3	8	0	0

DISCUSSION

Tables 3 to 7 give in detail the data obtained from hatches A to E, inclusive.

Beginning with hatch B, incubator 3, the results of incubation of the control eggs are incorporated in the tabulations for that and subsequent respective hatches, except incubator 2, hatch C, for which a control incubator was not available.

It will be noted from Tables 8 and 9 that among the n. e. chicks the hatchability in incubators was 82 per cent, and under hens 72 per cent, while the mortality was 14 per cent as compared with 11 per cent for hen-hatched chicks. In both groups *Salmonella pullorum* was recovered in 57 per cent of chicks dying within 14 days.

Among the b. w. d. chicks, hatchability in incubators was 75 per cent and under hens 61 per cent. Mortality of b. w. d. chicks in incubators was 27 per cent and under hens 8 per cent. In incubator-hatched chicks *Salmonella pullorum* was recovered in 81 per cent of chicks dying within 14 days, while in hen-hatched chicks recovery of the organism occurred in 75 per cent of cases.

Among the normal controls, no cases of *Salmonella pullorum* developed either in the incubator-hatched or in the hen-hatched chicks. This is considered the more significant in view of the fact that all the hens used in these hatching experiments were reactors to the agglutination test for *S. pullorum* infection and were taken from among the flock used to supply the infected eggs.

In Table 10 there is presented a summary of the results obtained from hatches A to H in agitated-air incubators and from hatches A to E in still-air incubators. It was originally intended that hatches F, G, and H in agitated-air incubators should be part of another

experiment, rather than being included in the experiments in incubator transmission of pullorum disease. However, inasmuch as these carefully controlled data were available, they were included in this report. This explains the three additional hatches taken from agitated-air incubators.

TABLE 10.—*Comparative results of hatches carried on in agitated-air and still-air incubators*

HATCHES A, B, C, D, E, F, G, H—AGITATED-AIR INCUBATORS

Eggs, kind	Eggs set	Fertile eggs	Chicks					
			Hatched		Died		Dead from which Salmonella pullorum was recovered	
	Number	*Number*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
N. e.	1,600	1,346	923	69	238	26	185	78
B. w. d.	1,492	1,194	704	66	417	53	363	87
N. c.	773	618	463	75	25	5	0	0

HATCHES A, B, C, D, E—STILL-AIR INCUBATORS

	Number	*Number*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
N. e.	833	701	438	62	76	17	37	49
B. w. d.	827	665	352	53	222	63	196	88
N. c.	418	343	235	69	26	11	0	0

Figures 4, 5, and 6 present in graphic form the average percentages of hatchability, mortality, and transmission of *Salmonella pullorum*

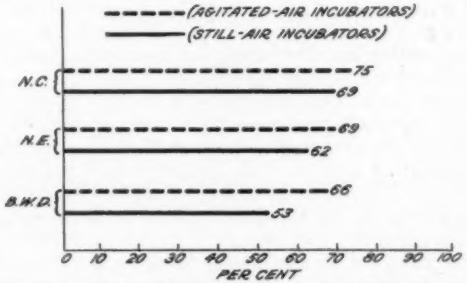

FIGURE 4.—Comparative percentages of hatchability of n. c., n. e., and b. w. d. infected eggs in agitated-air and in still-air incubators

infection, respectively, for hatches A to H in agitated-air incubators and hatches A to E in still-air incubators.

While it is possible that under other conditions the results reported in this paper might not be precisely duplicated, the writers feel that their experience as here reported, together with the results reported by Hinshaw and others is sufficient to show that pullorum infection will spread in incubator. It is a dangerous practice to set normal eggs in the same incubator with infected eggs, regardless of the type of incubator used.

SUMMARY AND CONCLUSIONS

Pullorum-disease infection was transmitted from diseased chicks to healthy chicks by exposure in an incubator for 18 to 24 hours from the time of hatching, without actual contact between the chicks.

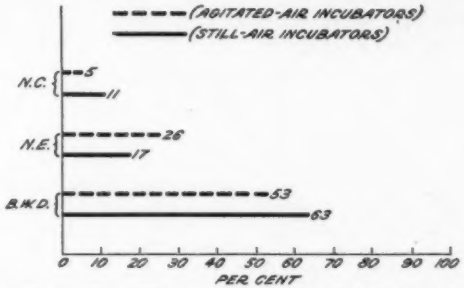

FIGURE 5.—Comparative percentages of mortality of chicks hatched from n. c., n. e., and b. w. d. infected eggs in agitated-air and in still-air incubators

A large percentage of chicks so exposed succumbed to the disease even under the most favorable subsequent brooding conditions.

There appeared to be a seasonal variation in the death rate due to this disease, the losses being greatest during the height of the laying season.

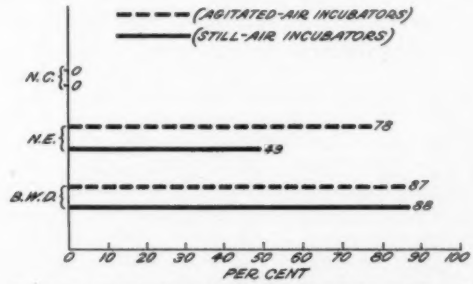

FIGURE 6.—Comparative percentages of transmission of pullorum disease in chicks hatched from n. c., n. e., and b. w. d. infected eggs in agitated-air and in still-air incubators

Hatchability of eggs in incubators was higher than under hens, but livability of hen-hatched chicks surpassed that of incubator chicks. The transmission of pullorum disease among incubator-hatched chicks was about the same as among hen-hatched chicks.

INHERITANCE OF THE SECOND FACTOR FOR RESISTANCE TO BUNT, TILLETIA TRITICI, IN HUSSAR WHEAT [1]

By F. N. Briggs [2]

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INTRODUCTION

In an earlier publication (*2*) [3] the writer presented data which indicated that Hussar wheat possesses two factors for resistance to bunt, *Tilletia tritici* (Bjerk.) Wint. One factor was shown to be the same as the dominant factor present in the Martin variety. The exact effect of the second factor could not be stated definitely at that time because there was some conflict in the evidence. The F_2 data from the cross between Hussar and Hard Federation indicated that the second factor for resistance allowed bunt to develop on about one-half of the heterozygous plants, but the F_3 data suggested that this factor was completely dominant like the factor present in Martin.

In the former paper it was pointed out that the effect of the second factor could be determined by isolating selections possessing the second factor but lacking the factor common to Martin and Hussar. Such a selection has been isolated, and data covering the inheritance of the second factor are now available.

REVIEW OF THE LITERATURE

Much of the literature pertinent to the inheritance of resistance to bunt was reviewed in the earlier publication mentioned above (*2*). Gaines (*4*) in 1925 described his extensive investigations on bunt resistance. He grouped varieties into four classes; namely, susceptible, intermediate, resistant, and immune. Crosses between susceptible varieties produced only susceptible offspring. Intermediate varieties crossed together produced both intermediate and susceptible progeny. When resistant varieties were crossed with susceptible ones, susceptibility was dominant, with only about 2 per cent of the progeny as resistant as the resistant parent. When susceptible varieties were crossed with the immune ones—Martin, Hussar, and White Odessa—there seemed to be a dominance of resistant plants. More than half of the hybrids in the third and subsequent generations produced less than 5 per cent of bunted heads, whereas about 20 per cent were bunt free. In crosses between immune and resistant varieties an occasional segregant showed more susceptiblity than the resistant parent. Gaines believed that the results indicated that immunity or resistance to bunt is due to the combined effect of several unit factors which, when

[1] Received for publication May 28, 1929; issued February, 1930. Cooperative investigations of the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Division of Agronomy, California Agricultural Experiment Station.

[2] The writer acknowledges valuable suggestions from Dr. R. E. Clausen, Division of Genetics, Prof. W. W. Mackie, Division of Agronomy, University of California, and from various members of the Office of Cereal Crops and Diseases.

[3] Reference is made by number (italic) to "Literature cited," p. 232.

all are present as in immune varieties, produce apparent dominance, but a lesser number would give the recessive effect. On the other hand, the writer (*2*) found that Martin has one dominant factor for resistance, whereas Hussar has the same factor that Martin has and probably one other.

Gaines and Singleton (*6*) reported that "Marquis is fairly susceptible when fall sown, but does show a small amount of resistance in that three of the four replications were in the 70.5 per cent class. Marquis, when spring sown, falls into the 8 per cent class. Turkey, which falls into the 8 per cent class if fall sown, is immune in the spring sowing." An analysis of F_3 families from a cross of Marquis × Turkey showed some segregates more susceptible and others more resistant than the parents. The factor for resistance in Turkey was thought to be about four times as "prepotent" as the Marquis factor. A correlation coefficient of 0.711 ± 0.027 between fall-sown and spring-sown rows indicated the action of the same genes, the spring conditions appearing only to intensify their expression.

METHODS AND MATERIALS

The parental material and hybrid populations were grown in the field at University Farm, Davis, Calif. Conditions there favor such investigations because relatively high bunt infection can be obtained when wheat is sown in the fall. Both spring and winter varieties may be seeded at that time without any danger of winterkilling and with the assurance that both types will mature in the following summer.

The seeds were thoroughly blackened with bunt by placing an excess quantity of the spores with the wheat in a glass container and shaking it vigorously. The inoculum, *Tilletia tritici*, was collected by W. W. Mackie in 1917 on Little Club wheat in the Montezuma Hill district of Solano County, Calif. This was originally propagated by Mackie on the Little Club variety of wheat in the botany garden at Berkeley, Calif. Since 1919 the writer has propagated smut from this same collection on White Federation wheat at Davis. The inoculum used, therefore, has been derived from one original collection of bunt. At first this procedure was followed not because it was suspected that there were physiologic races of bunt, but because this collection method offered an accessible and definite source of spores. More recently, Faris (*3*), Rodenhiser and Stakman (*8*), Reed (*7*), and Gaines (*5*) have reported physiologic forms of this fungus. The fact that the same collection of bunt has been used continuously at Davis makes it reasonably certain that the same form or mixture of forms has been employed in all the writer's investigations. This is indicated also by the consistent way in which the parental wheat varieties have reacted to this inoculum.

The wheat seeds were spaced from 2 to 3 inches apart in rod rows 1 foot apart. The entire nursery was sown within three or four days in order to avoid the effects of different temperatures and soil moistures. The nursery always was sown in a field where no wheat had been grown during the previous year, so that it was almost entirely free from volunteer grain.

At harvest time the plants in each row were pulled and separated into two piles, bunt free and bunted. The total number of plants and the number of bunted plants were recorded, and the percentage

of bunt infection was calculated. A plant was classified as bunted if it showed any infection.

Hussar was one of two varieties of wheat found to remain free from bunt when inoculated with spores of *Tilletia tritici* in an extensive test conducted by the Washington, Oregon, and California agricultural experiment stations in cooperation with the Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture (*9*). Hussar has been entirely free from bunt over a 9-year period when inoculated with the bunt collection used in these experiments. It has been attacked by some collections of bunt used elsewhere (*5*, *7*). Little Club, the susceptible variety used in these experiments, has produced from 70 to 95 per cent of bunted plants when the same inoculum was employed that produced no bunt in Hussar.

A strain lacking the factor for resistance common to Hussar and Martin, but having the second resistance factor carried by Hussar, was isolated from a cross between Hussar and Hard Federation. Twenty resistant F_4 plants of this cross were crossed with Martin and tested for bunt resistance in the F_2. The progenies from plants numbered 1403, 1418, and 1511 of the Hussar × Hard Federation cross contained about 7 per cent of bunted plants, which indicated that these plants lacked the Martin factor. The other 17 progenies did not segregate, indicating that here both parents carried the Martin factor for resistance. At the time the parent F_4 plants were crossed with Martin they were crossed also with Little Club to supply material for a genetic study of resistance to bunt.

Unlike Hussar, selection 1418 is not immune from bunt, but it is highly resistant. Four rod rows produced 0.95 per cent of bunted plants in 1927, and 13 rows averaged 1.7 per cent in 1928. The fact that a little bunt appeared in this selection might suggest that this factor is not so effective in controlling bunt as is the Martin factor. Such, however, may not be the case, for these plants were from material which had been selected the previous year from rows with a slight percentage of bunt, and they were being grown to determine whether this percentage might not be due to modifying factors. The selection 1403 has been entirely bunt free. This selection would have been used in the study here reported, had not the cross of it with Little Club failed to yield seed because of an accident. The slight percentage of bunt in selection 1418 has not disturbed the results materially.

EXPERIMENTAL RESULTS

F_2 PROGENIES

All F_2 progenies were grown in 1927. The resulting data are presented in Table 1.

TABLE 1.—*Total number of plants and number and percentage of bunted plants in parents and F_2 of the crosses named*

Parent or cross	Number of plants		Percentage of bunted plants
	Total	Bunted	
Selection 1418	210	2	0.95
Little Club	242	202	83.50
Selection 1418 × Little Club	762	406	53.30
Selection 1418 × Martin	81	6	7.40

In the F_2 progeny of selection 1418×Little Club there were 53.3 per cent of bunted plants, as compared with 19.2 per cent in the F_2 of Martin×Hard Federation and 17.2 per cent in Martin×White Federation (*2*). No doubt some susceptible plants escaped infection. On the other hand, some resistant plants probably became infected because selection 1418 is not immune. The data therefore indicate that the factor for resistance in selection 1418 allows bunt to develop on about 50 per cent of the heterozygous plants.

In the F_2 progeny of selection 1418×Martin there were 7.4 per cent of bunted plants, as compared with 9.7 per cent in both Hussar×Hard Federation and Hussar×Baart (*2*). However, there were only 81 plants as compared with about 2,000 in each of the Hussar crosses. The data suggest that Martin and selection 1418 contain the two main factors for immunity and resistance present in Hussar.

SEGREGATION IN F_3

Data collected in F_2 indicate the genetic segregation only roughly, because some susceptible plants apparently escape infection. In the most susceptible varieties it is rare that 100 per cent of the plants in a rod row are infected. If seed of the disease-free plants of such a susceptible variety is selected and sown the following year, the resulting crop does not differ from the unselected variety in resistance to bunt. F_2 data are valuable, however, because they give some indication of the number of factors for resistance present. They also indicate the percentage of bunt to be expected in heterozygous F_3 rows.

In 1928 there were grown 300 F_3 rows of selection 1418×Little Club from seeds of 300 F_2 plants not subjected to bunt infection in F_2, and 81 F_3 rows of selection 1418×Martin. The results are recorded in Table 2.

TABLE 2.—*Distribution of the rows of the parents and of the F_3 hybrids named into percentage classes for bunt infection*

Parent or cross	Distribution of rows by percentage classes for bunt infection																				Total number of rows
	0	<5	5–10	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	85–90	90–95	
election 1418	6	6	1																		13
Little Club																	2	1	4	1	8
Selection 1418×Little Club	23	31	11	8	1	6	8	14	15	27	31	27	15	8	12	9	15	19	12	8	300
Selection 1418×Martin	34	10	11	4	6	5	2	2	1	0	0	2	1	1	0	1	0	1			81

The distribution of the rows of selection 1418×Little Club into percentage classes of bunt infection is shown by the solid line in Figure 1. This may be compared with the distribution of Martin×White Federation, which is shown by the broken line and which is reprinted from the earlier publication (*2*, *fig. 2*). The class distributions are given in percentages because of the difference in size of the two populations.

The curve for the cross selection 1418×Little Club is distinctly trimodal. The distribution of rows under the three modes agrees very closely with a 1 : 2 : 1 ratio. The first mode represents 74 rows where 75 were expected. The second mode represents 147

rows where 150 were expected, and the third mode represents 79 rows where 75 were expected. The deviations between the obtained and calculated numbers are very small. However, the two minimums on the curve should not be thought of as being the exact limits between the three phenotypes.

The spread of each group exceeds that which at first might be expected. The resistant parent 1418 did not show percentages of bunted plants beyond the 5 to 10 per cent class, while the resistant hybrid rows, as set off by the first minimum, include percentages up to 15 to 20 per cent. The 15 rows of F_2 plants grown in 1927 contained from 31.7 to 66 per cent of bunted plants, while the heterozygous F_3 rows ranged from 17.5 to 62.5 per cent. Finally, the rows of the susceptible parent contained from 77.5 to 92.5 per cent of bunted plants, as compared with 62.5 to 92.5 per cent for the susceptible F_3 rows. If larger numbers of parent rows had been grown, it is entirely

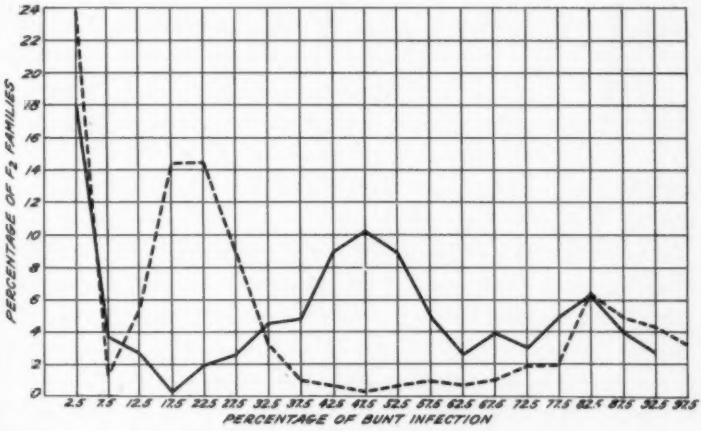

FIGURE 1.—Distribution of F_2 families of the crosses selection 1418×Little Club wheat (solid line) and Martin×White Federation wheat (broken line) into 5 per cent classes for bunt infection on the basis of the percentage of infection occurring in the F_2 rod rows

possible that the extremes would have approached those of the hybrid rows. Data to be published elsewhere show that modifying factors may be present. The presence of modifying factors would increase variability, as would the presence of field hybrids or occasional mechanical mixtures which might have occurred in threshing and seeding. Considering all variations, the data indicate that selection 1418 differs from Little Club in one main factor for resistance to bunt.

If the resistance factor of selection 1418 and the Martin factor are the only factors determining resistance to bunt in Hussar, a cross between selection 1418 and Martin should give results similar to those obtained by crossing Hussar with a susceptible wheat. Such a cross was made, and small F_2 and F_3 populations were grown. In F_2, as shown in Table 1, 7.4 per cent of bunted plants occurred, as compared with 9.7 per cent in both Hussar×Baart and Hussar×Hard Federation (*2*). The distribution of F_3 rows by percentage classes for

bunt infection is given in Table 2. This distribution is similar to that obtained when Hussar was crossed with Baart (*2*), as may be seen from Figure 2. For comparison, this latter curve is reprinted from the earlier publication (*2, fig. 4*).

With two factors for reaction to bunt present, it is difficult to determine the limits of the phenotypes, because they overlap, due to the variable amount of bunt produced in a single genotype. However, the susceptible and the resistant rows may be determined fairly accurately. If the rows containing from 50 to 85 per cent of bunt are considered as homozygous susceptible, there are six strains where five were expected. Defining the susceptible group as one containing 60 to 95 per cent of bunt, to correspond with the susceptible group in

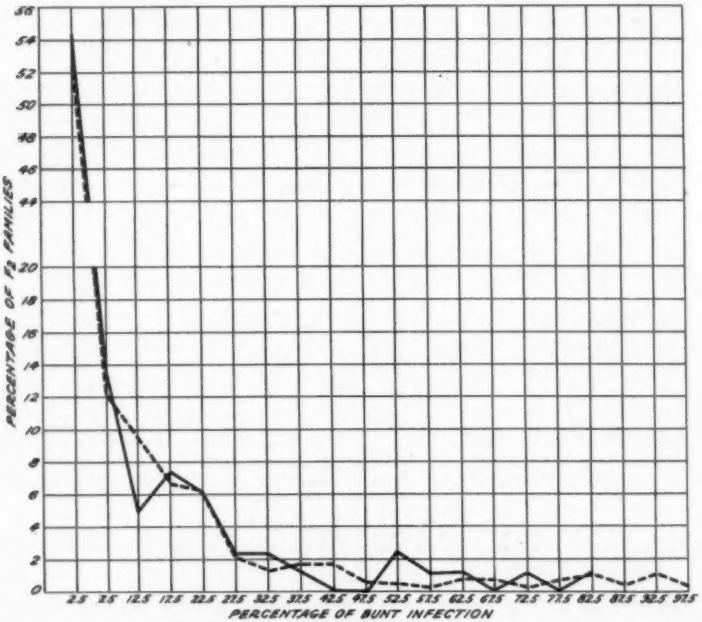

FIGURE 2.—Distribution of F_2 families of the crosses selection 1418×Martin wheat (solid line) and Hussar×Baart (Early Baart) wheat (broken line) into 5 per cent classes for bunt infection on the basis of the percentage of infection occurring in the F_3 rod rows

the cross 1418×Little Club, gives three susceptible rows where five were expected. This latter is a deviation of 2 ± 1.47, which is a satisfactory agreement. There are 34 smut-free rows where 35 were expected. Past experience indicates that the resistant group should not be made up entirely of smut-free or completely resistant rows, but should include some rows with a low percentage of bunt. Considering that only 81 rows were available as compared with 598 from the cross Hussar×Baart, the similarity between the curves is close. The data therefore show that the Martin factor and the resistance factor in selection 1418 are the two factors that cause resistance in Hussar, at least so far as the smut collection used in these experiments is concerned.

DISCUSSION

The resistance of cereals to smut may be due to multiple genetic factors in some cases. Wakabayashi (*10*) concluded that the resistance of Red Rustproof oats to covered smut, *Ustilago levis*, depended on three independent dominant factors. Barney (*1*) also found three independent dominant factors in oats for resistance to loose smut, *U. avenae*. Gaines (*4*) believed that resistance to bunt, in wheat varieties such as Martin, Hussar, and White Odessa is due to multiple factors.

Under the present conditions for studying the inheritance of resistance to bunt, the presence of multiple factors for resistance in a variety may make it impossible to determine with certainty the number of factors present and the exact effect of each. Because of the variability of the percentage of bunt in strains of the same genotype, there is likely to be an overlapping of phenotypes where two or more factors are present, with the result that it is impossible to establish the limits of the various phenotypes. Until methods are developed whereby 100 per cent of the susceptible plants become diseased, so that the genetic analysis may be made in F_2, it may be necessary to isolate each factor for reaction to bunt and to study it alone.

Hussar differs from the susceptible varieties of wheat in two main factors for resistance to the particular collection of bunt used in these experiments. One of these is the same as the completely dominant factor in Martin, and the other allows the disease to develop on about 50 per cent of the heterozygous plants. This does not preclude the possibility of still other factors which might become apparent in the presence of other physiologic forms of bunt.

The factor for resistance in Martin hereafter will be designated as *MM*. The second factor in Hussar, exemplified in selection 1418, will be designated as *HH*. Martin then may be designated as *MMhh* and Hussar as *MMHH*.

Some progress has been made in the production of bunt-resistant wheats, and through continued breeding more resistant commercial varieties should become available within the next few years. As physiologic races of bunt exist, it seems very desirable to isolate and study the effect of as many different factors for resistance as possible. The more factors for resistance the plant breeder has at his disposal, the more likely is he to succeed in breeding bunt-resistant wheats in the presence of two or more physiologic forms of this disease organism.

SUMMARY

In a previous paper data on the inheritance of resistance to bunt in crosses with Martin and Hussar wheats were presented. The data available at that time showed that Hussar had the same dominant factor for resistance as the one found in Martin and probably also had one other. The exact effect of the second Hussar factor was not determined.

By appropriate breeding tests there was secured selection 1418, which contained the second Hussar factor but did not have the factor for bunt resistance common to Martin and Hussar.

Selection 1418 was crossed with susceptible Little Club, and the inheritance of resistance to bunt was studied in F_2 and F_3. This

selection was found to differ from Little Club in one main factor for resistance to bunt. Unlike the completely dominant Martin factor, it allows about 50 per cent of the heterozygous plants to become infected.

Martin crossed with selection 1418 segregated in F_2 and F_3 similarly to Hussar×Baart, showing that the factors in Martin and in selection 1418 are the two main factors for resistance to bunt in Hussar.

The Martin factor for resistance hereafter will be designated as *MM*, and the second Hussar factor as *HH*. Martin then may be designated as *MMhh* and Hussar as *MMHH*.

The isolation and study of different factors for resistance to bunt should aid in the breeding of resistant wheats where different physiologic forms of this disease organism exist.

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AN AUTOMATIC WATERING SYSTEM WITH RECORDER FOR USE IN GROWING PLANTS[1]

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INTRODUCTION

It is often highly desirable to use automatic balances in studies of water relations in plants. There have appeared at different times various kinds of apparatus designed to keep the soil-moisture content constant and to record the amount of moisture lost by plants. The types of apparatus described,[2] as a rule, have not possessed both these features, or have been inaccurate apparently due to the effects of wind or temperature.

The apparatus described in this paper was designed for use in connection with studies now being carried on in the Office of Tobacco and Plant Nutrition on the response to length of day of plants at constant temperature and humidity; in these studies close control and accurate records of soil moisture are necessary. The system is mechanically somewhat similar to the apparatus used by Blackman and Paine [3] and Hamorak [4] and is adapted both to the control of soil moisture and to the recording of moisture used; it should prove as serviceable in the field as it has in the control room. It has been in operation for about two years and even in its original form has proved reliable and required but a few minutes' attention every day or two.

The essential component of the apparatus is a swinging or rocking funnel through which a stream of water flows at a constant rate. This stream of water is diverted into a plant container for almost exactly one minute whenever the loss in weight of the container causes the beam of the balance on which it rests to close an electrical contact. The same current that operates the funnel also moves a magnetically operated pen over a moving time chart, thus producing a permanent record. The quantity of water added is then easily computed, since it is equal to the flow of water per minute multiplied by the number of times the funnel has been actuated as indicated by the chart.

APPARATUS

The system may be said to comprise the following units, each of which will be discussed in turn: (1) A minute-contact master clock,

[1] Received for publication July 1, 1929; issued February, 1930.

[2] BURGERSTEIN, A. DIE TRANSPIRATION DER PFLANZEN. EINIGE PHYSIOLOGISCHE MONOGRAPHIE. 3 t., illus. Jena. 1904–25.

BRIGGS, L. J., and SHANTZ, H. L. AN AUTOMATIC TRANSPIRATION SCALE OF LARGE CAPACITY FOR USE WITH FREELY EXPOSED PLANTS. Jour. Agr. Research 5: 117–132, illus. 1915.

LIVINGSTON, B. E., and HAWKINS, L. A. THE WATER-RELATION BETWEEN PLANT AND SOIL. 48 p., illus. Washington, D. C. 1915. (Carnegie Inst. Wash. Pub. 204.)

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(2) a 30-volt storage battery with combined charging and timing panel, (3) a rocking funnel, (4) a balance, (5) a recorder, and (6) a soil-moisture distributor.

The minute-contact master clock employed has an accuracy, under normal room conditions, of ±20 seconds per month and closes an electrical contact, each minute on the minute, for about two seconds. However, as a clock error of even 14.4 minutes per month would correspond to an error of only 1 per cent in the quantity of water added, almost any fairly accurate clock having a minute contact can be used. If necessary, a clock having three hands can be converted into a suitable timing device by causing the second hand to dip into a mercury

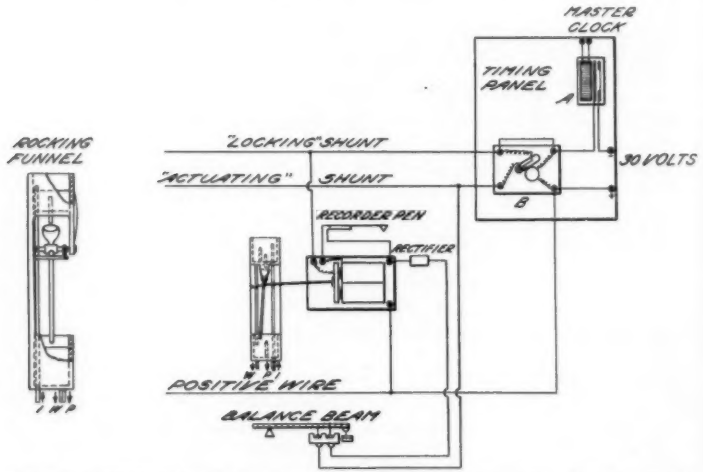

FIGURE 1.—Electrical circuits of a timing panel with one rocking funnel and balance. To the left is a side view of a rocking funnel and mounting comprising a water level with drip tube on top, support for pivoted shaft underneath, and double catch cup at the bottom. *a*, An intermittent relay operated by the minute-contact clock; *b*, mercury-vacuum relay; *i*, water inlet; *p*, outlet into plant container; *w*, waste

well once during each revolution, or by placing a cam on the second-hand shaft, which in turn opens and closes an electrical contact.

The combined charging and timing panel is shown in Figures 1 and 2. The former, with a 30-volt storage battery on trickle charge, is provided with a safety relay that opens when the line current fails. A specific description of its details is unnecessary, as they are standard and can be found in many good electrical textbooks. The use of storage batteries is considered advisable only in order to prevent interruptions due to line failure.

The timing panel consists of relays *A* and *B*, the former controlling both the "actuating" and the "locking" shunt wires to the "funnel" relays, and the latter the actuating shunt wire only. Relay *A* is an intermittent relay operated by the minute-contact clock. It alternately opens and closes each minute on the minute; that is, its contact is open for one minute, then shut one minute, then open one minute, and so on. Relay *B* is a mercury-vacuum switch having a somewhat greater time lag than relay *A*. It is operated by relay *A*,

the closing of relay *A* resulting in the opening of relay *B*. The circuit through the actuating shunt is, therefore, closed only each alternate minute for a length of time corresponding to the difference in lag of the relays, since the actuating shunt circuit must be completed through the contacts of both relays.

It will be noticed that the positive wire from the battery goes to the lower coil post of each telegraph relay rocking the funnels. The current passing through the coils of these funnel relays can flow back to the battery through either the actuating or the locking shunt. Since the beam contact is in the actuating-shunt line, the closing of the funnel relays can occur only during the instant at the beginning of each alternate minute when relay *A* contact is closing and relay *B*

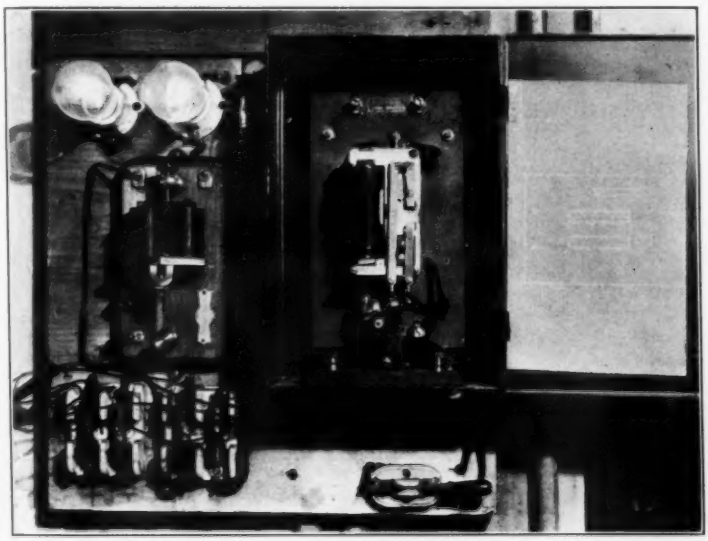

FIGURE 2.—Combined battery trickle charger with safety relay and timing relays

contact has not yet opened. Once the funnel relay is closed, however, the current passes through the funnel-relay contact and the locking shunt and locks or keeps the funnel relay closed until relay *A* contact opens at the end of the minute.

In order to prevent the feeding back of current to other watering units on these shunts, a kuprox or other dry rectifier unit, such as is used in battery charging on alternating current, is placed in series with each balance-beam contact. The use of spring contacts opened by the funnels, the method originally employed, was abandoned in favor of these units, which require less attention; that is to say, there is one contact less per automatic balance to require attention.

The rocking-funnel mounting (figs. 1 and 3) consists of a single brass casting, the top of which serves as a constant water level (1 inch deep) from which water flows at a fixed rate through a vertical vent or drip tube into the funnel suspended underneath. The inlet to the drip tube should be at least a quarter of an inch above the

bottom of the constant water level. The funnel passes through a hole in a pivoted shaft and is biased by means of a spring. With the rocking funnel in the position shown in Figures 1 and 2, the water flows into the left-hand compartment of the catch cup and the waste runs out through tube *w*. In the event, however, that the container with its soil and plants loses weight, the beam contact of the balance on which it rests is closed, thus permitting the funnel relay to be closed during the instant the actuating-shunt circuit is completed through relay *a* and relay *b* contacts. The funnel relay at the same time closes its contact to the locking-shunt line and is thus prevented from reopening until relay *a* opens at the end of the minute. Closure of the funnel relay causes the funnel outlet to swing to the right and

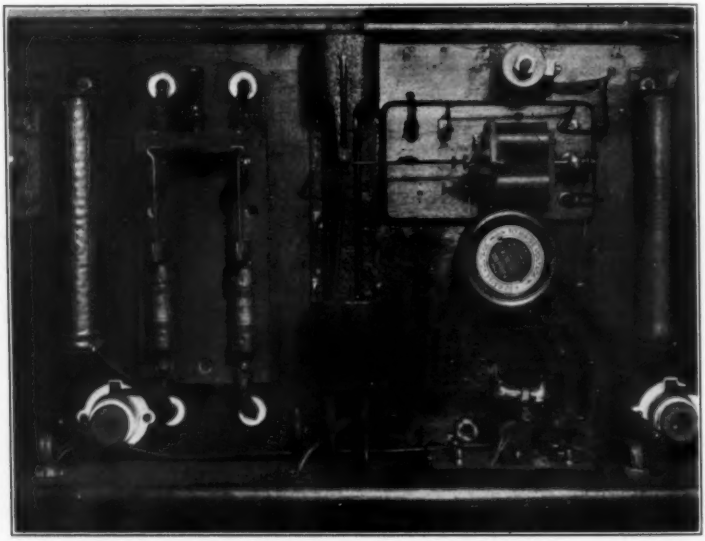

FIGURE 3.—Rocking funnel and relay as they appear on switchboard above light case. The rectifier unit, here functioning as a check valve, is just above the relay

empty into the right-hand compartment of the catch cup, whence the water flows through tube *p* into the plant container. Tube *i* is the water inlet to the water level at the top.

The balance contact consists of a nichrome wire U whose bend is twisted about another wire passing around the beam and firmly fastened to it. It is insulated from the beam, however. This U, or double contact, dips into two iron cups filled with mercury and supported in a fiber block that is bolted to the frame of the balance.

As designed, this system of automatic watering can readily be used with a recorder. The recorder should preferably be what is known as a time recorder[5] and is used only to record the times at which the funnels are rocked. Since, however, the water presumably is

[5] Since this paper was written, small, inexpensive ratchet counters have been found satisfactory when readings at regular intervals are practicable. These counters are actuated mechanically by the swing of the rocking funnel.

flowing at a constant rate and flows into the plant container for exactly one minute each time the funnel is rocked, the quantity of water added is equivalent to the number of times the funnel has been rocked multiplied by the water flow per minute.

Suitable recorders are being produced commercially or can be assembled with a moving chart and magnet pens. A recorder having pen coils of 30 ohms for use on 3 volts should prove satisfactory for use in series with 250-ohm funnel relays on 30-volt battery. Three typical 24-hour records at varying rates of water flow are shown in Figure 4.

In the event that a recorder is not required, the system can be greatly simplified, since in this case only the rocking funnels with their relays and the balances will be necessary.

METHOD USED TO DISTRIBUTE THE WATER THROUGH SOIL IN THE PLANT CONTAINER

If 80 pounds of wet soil per container are used, the theoretical degree of soil-moisture control with a balance sensitive to 1 ounce is ± 0.04 per cent soil moisture on the wet basis. Ignoring the effect of increasing mass of the plant, this means that about 1.7 cubic inches of water, or 1 ounce, must be distributed through a volume of about 2,000 cubic inches; or, if only horizontal uniformity is considered, the 1.7 cubic inches of water must be spread over a soil area of 231 square inches (container about 16 by 16 inches at base) to a thickness of 0.0008 inch. Even if feasible, an absolutely uniform distribution of water throughout the soil mass would probably require costly and complicated apparatus. Therefore, like previous investigators, the writer decided to attempt only horizontal uniformity. This is, after all, the natural distribution of moisture in the soil of the field, although the objection might be raised that the roots of the plants will tend to grow into the soil level having the most favorable moisture content instead of scattering uniformly through the soil; that is, the roots are not entirely free from any influence caused by variation in soil moisture. Within certain limits the plants are not growing in soil of average moisture content represented by the whole vertical soil column.

Uniform horizontal distribution of the soil moisture was accomplished by adding the water through a copper tube of ¼-inch outside diameter perforated at 2-inch intervals. The tube was bent into an 8-inch square with two vertical risers used as inlet tubes. These risers, which were not perforated, projected above the soil. The tubes were cleated to the bottom of the plant container, covered with a half inch of sand saturated with water, and the soil added to within about 1 inch of the top. Moisture determinations made at different times in vertical soil columns were within ± 1 per cent throughout the containers.

Evaporation from the plant containers is minimized as follows: The plant containers used in the present case are ¾-inch cypress (outside 17 by 17 inches by 10½ inches high), covered on the inside with asphalt applied hot and with two coats of white enamel on the outside. The lids, which are of galvanized iron, are coated on the inside with asphalt applied hot and on the outside with two coats of white enamel. There are altogether fifteen 2-inch holes and two 5/16-inch holes in each lid. The latter are made air-tight

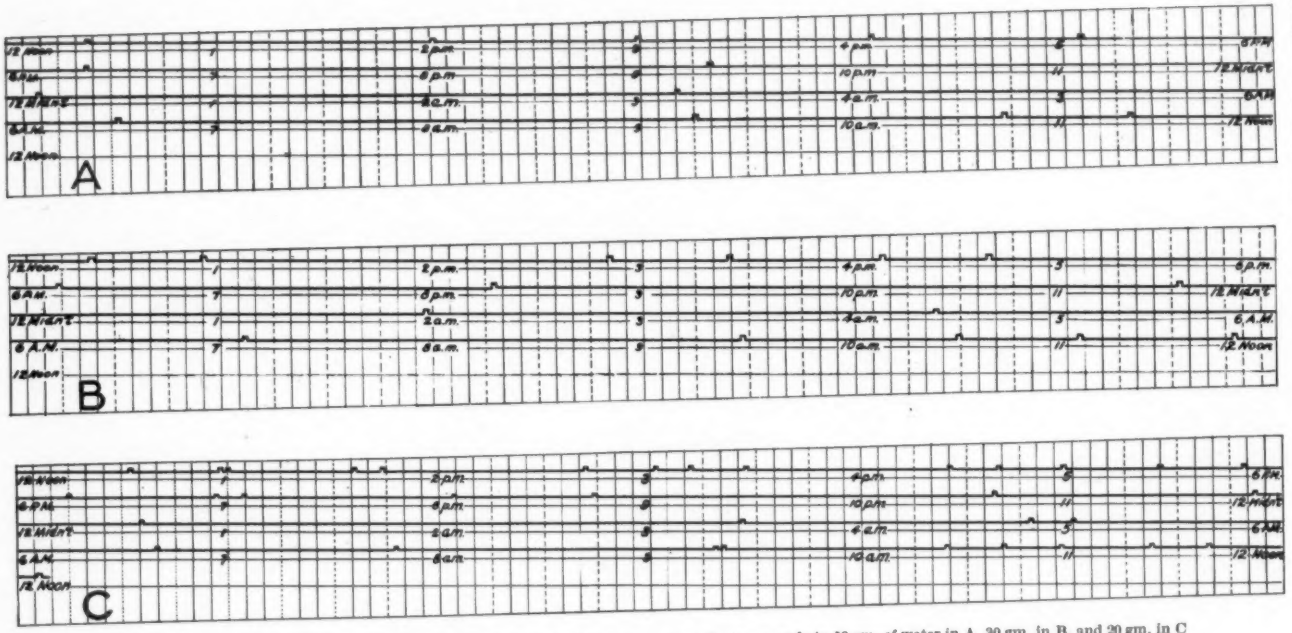

FIGURE 4.—Twenty-four-hour charts with transpiration records. Each tooth or notch corresponds to 50 gm. of water in A, 30 gm. in B, and 20 gm. in C

with rubber tubing around the risers from the watering tubes. The former are provided with perforated split paraffined corks. The corks are placed around the stems of the plants and can be packed with cotton to prevent loss of moisture. Evaporation from a container covered with a lid and unparaffined solid corks was 6,050 gm. when kept in a greenhouse from March 2, 1927, to January 11, 1928 (315 days), or less than 1 ounce a day.

Evaporation from a container at $77.0° \pm 0.5°$ F. and 54.2 ± 0.75 per cent relative humidity under continuous artificial illumination of about 4,500 foot-candles with a Mazda lamp screened by 3 inches of water and with an air velocity of about 90 feet per minute amounted to 126 gm. per day or 5.26 gm. per light hour. A similar determination in the dark gave 3.98 gm. per unlighted hour. The holes in the split corks were left open in both cases.

DISCUSSION

The expectation of accuracy in results depends largely upon balance sensitivity for average soil moisture and upon the rate of water flow for transpiration or evaporation requirements. As has been noted, the balance sensitivity with the weights of soil used would theoretically provide for maintaining average soil moisture constant within a variation of ± 0.04 per cent with water flow of 1 ounce per minute. This is easily checked by observation of the balance beam. Its position should at all times indicate an exact tare of the plant container, so that the removal of an ounce of material from the container will throw in the balance contact.

Accuracy of transpiration records is largely dependent upon the accuracy with which each record on the chart represents a definite amount of water. The physical factors governing flow in small tubes and nozzles are known,[6] as is also the fact that deposition of sediment gradually reduces the rate of flow. Head is maintained constant by use of a water level with a large capacity overflow, and temperature by thermostatic control of the water temperature to $\pm 1.5°$ C. While sedimentation could be minimized or eliminated by the use of distilled water or of one of the well-known methods of water softening, these methods are costly and require considerable attention. It therefore seemed more practicable to determine, if possible, the conditions under which clogging is least and to clean the drip nozzles as necessary.

When the tap water in Washington, D. C., is used, clogging is believed to be due to the deposition upon the walls of the drip tube of colloidal material from the previously clear water. At least it was found that a strainer having holes one-fourth the bore of the drip tube would not retain the sediment. Briefly summarized, it appeared that the apparent self-cleaning action of the drip tube was greatest when the type of flow was intermediate between drops and streamline or even flow. Constancy between duplicate tests of the rate of flow is satisfactory, and although not so good as with streamline flow it is much better than when the flow is in drops. Maximum variations between duplicates at the rates studied in grams per minute were: (1)

[6] MARKS, L. S. MECHANICAL ENGINEERS HANDBOOK. PREPARED BY A STAFF OF SPECIALISTS. Ed. 2, p. 281. New York and London. 1924.

16.15 ± 0.81 per cent for drops; (2) 20.20 ± 0.50 per cent for flow intermediate between drops and streamline flow; (3) 29.91 ± 0.57 per cent for flow as in 2; and (4) 49.80 ± 0.14 per cent for streamline flow. Decrease in rate of flow with time was found to be, respectively, as follows: (1) Inconsistent; (2) 0.93 per cent in 7 days (0.99 per cent variation between maximum and minimum during this interval); (3) 0.38 per cent in 15 days (1.09 per cent variation between maximum and minimum during this time); and (4) 1.08 per cent in 2 days. It is probable that it is entirely practicable to obtain a water flow and therefore transpiration records accurate to ± 0.5 per cent or better by an inexpensive method requiring very little attention. It is necessary to control the temperature of the water and to adjust for head of water and length, bore, and taper of the drip nozzle to obtain the proper type of flow. The drip nozzles should be cleaned when necessary by means of a soft copper wire slightly smaller than the bore of the drip.

The data below are given to permit duplication, and to give an idea of the relations between the dimensions of the drip nozzle and the rate of water flow. The drip tubes were made of $\frac{5}{16}$-inch brass rod threaded for 1 inch and slotted at the top to permit adjustment for head of water. They were 1¼ inches long with untapered nozzles. The inside diameters of the tubes, in the same order as the flows given in the preceding paragraph, were 0.042, 0.0465, 0.052, and one-sixteenth inch at heads measured from top of tube of thirteen-sixteenths, seven-eighths, one-half, and seven-sixteenths inch.

Time accuracy in addition of water and in its recording is dependent upon the elimination of beam vibration or oscillation and upon variations in sensitivity of the balance.

There is nothing to prevent the outdoor use of this system of automatic balancing for soil-moisture control and record. The most important variables thus introduced are wind and rain. The first necessitates the use of an oil dashpot mounted on the balance beam, as used by Briggs and Shantz,[7] to prevent oscillation, and the latter a shelter. This system has, indeed, two distinct advantages over former systems in that the actuating contact is very short, occurs at intervals of two minutes, and minimizes though it does not eliminate the effect of beam oscillation. If necessary, all the control apparatus can be centralized in a shelter and the balances scattered in a surrounding field. Also, with the type of balance described herein, that is, beam below pan, the protection of the balance against rain is very simply accomplished by inverting a shallow square tray on the platform of the scale with the lip of the tray just short enough to avoid touching the ground. A still more effective method would be to place the scale on a platform a few inches high and extend the lip of the pan to below the bottom of the scale.

The system herein described, while subject to refinements, has given very satisfactory results, even in its original form, for over two years, eight units being in operation simultaneously for periods of six weeks or more at a time. It requires a minimum of attention every day or two to insure accurate control of average soil moisture to ± 0.2 per cent and transpiration records accurate to ± 1 per cent

[7] Briggs, L. J., and Shantz, H. L. Op. cit., fig. 13.

or better. The balance beams should be looked at every few days to make sure that the containers are tared; the water temperature should be kept constant to 1° or 2° F., and a wire should be passed through the drip outlets emptying into the funnels at least once every second day. If records of less accuracy are sufficient, the temperature control can be less accurate, while the drip outlets may be inspected only two or three times a week, depending on the accuracy desired.

SUMMARY

A fully automatic watering system that can be used with a recorder has been described for use in studies of plant transpiration and growth when soil moisture must be kept constant and a record must be made of the water used. Average soil moisture can be controlled as accurately as necessary by the use of a balance of sufficient sensitivity and horizontal uniformity of soil moisture to ± 1 per cent, while transpiration records accurate to ± 1 per cent can easily be obtained. The system requires, barring accidents, not over five minutes' attention per unit every second day. It is suitable for use indoors or outdoors. It is flexible enough to be used in single or multiple units, with or without a recorder, and its component parts are comparatively inexpensive and easily available.

THE INFLUENCE OF THE POTASH CONCENTRATION IN THE CULTURE MEDIUM ON THE PRODUCTION OF CARBOHYDRATES IN PLANTS[1]

By GEORGE JANSSEN, *Assistant Agronomist*, and R. P. BARTHOLOMEW, *Assistant Agronomist, Arkansas Agricultural Experiment Station*

INTRODUCTION

The chief rôle assigned to potassium in plant nutrition is its function in the syntheis of sugars, starch, and possibly other carbohydrate compounds (*11*, *21*, *28*, *29*, *31*).[2] Its relation to the synthesis of proteins has also been discussed (*10*, *21*). That it is to a large extent involved in photosynthesis is shown by its presence in the green portions of leaves and stems (*4*, *23*, *29*). Stoklasa (*29*) has suggested a means by which the potassium ion may enter into the reaction of the photosynthetic process during the syntheis of sugars. He has also shown by chemical analyses that plants grown on soils low in potassium are low in total sugars and starch, while those grown on soils high in potassium are high in these compounds. Other workers report similar results (*11*, *28*, *33*). Dowding (*4*) has shown that all meristematic tissue of the spruce is particularly rich in potassium. Reed (*26*) found that potassium was necessary for germination and growth of certain mosses and for starch production in all the green plants which he studied. He further found that mitotic cell division never took place without a suitable supply of potassium.

OBJECT OF THE EXPERIMENT

Since it is a well-recognized fact that potassium performs a very important function in photosynthesis and in the formation of carbohydrates, either aiding in their synthesis or translocation, or both, it appeared important to learn what effect the gradual increase of potassium in the nutrient medium would have upon plant growth and upon the synthesis of carbohydrates. In a previous paper (*2*) it was pointed out that the percentage of potassium in plants increased with the amount of available potassium supplied. It was the object of the present investigation to determine to what extent increments of potassium in the plant, brought about by varying the amounts of available potassium in the nutrient medium, affect (1) the total dry weight per plant; (2) the percentage of dry matter; (3) the total percentage of sugars, starch, and hemicellulose; and (4) the total sugars and starch per plant or unit of plants.

PLAN OF THE EXPERIMENT

Plants which have been designated as high and low potassium feeders were used in these experiments; they were oats, cowpeas, soybeans, sweetclover, cotton, Sudan grass, and corn. These plants

[1] Received for publication Apr. 23, 1929; issued February, 1930. Approved by the director of the Arkansas Agricultural Experiment Station. Research Paper No. 167, Journal series, University of Arkansas.

[2] Reference is made by number (italic) to "Literature cited," p. 259.

were grown in water cultures, sand cultures, and field soil, to which increments of potassium were added. In some cases all three methods of culture were used for the same crop plant.

METHODS

In the nutrient-solution culture work two types of solutions were used. Freedom from the sodium ion and the ability of the plant to make good growth in the medium were the basis upon which the solution was selected. It is believed that the presence of sodium ions (*12*) in a nutrient to which potassium is added for the purpose of study may lead to erroneous conclusions. A discussion of this possibility was presented in another paper (*2*).

The nutrient solution used for the first series of soybeans and oats grown in sand (Tables 1, A, and B, and 5, A, and B) contained the following compounds: Solution 1: $Ca(NO_3)_24H_2O$, 147 gm.; $CaH_4(PO_4)_2H_2O$, 26.9 gm.; dissolve and make to 2,280 c. c. Solution 2: $MgSO_4.7H_2O$, 90 gm.; dissolve and make to 2,280 c. c.

Fifty cubic centimeters each of solutions 1 and 2 were added to 1 liter of water, giving a salt concentration of 0.58 per cent. The potassium was added from a stock solution of potassium chloride. The stock solution contained 1.91 gm. KCl per liter, equivalent to 1,000 mgm. potassium per liter, or 1,000 parts per million. Thus 1 c. c. of this solution diluted to 1,000 c. c. gave a concentration of 1 mgm. of potassium per liter, or 1 p. p. m.

The jars to which the nutrient solutions were added were provided with an outlet at the bottom (modified after the method of McCall (*22*), to which suction could be applied, thus facilitating the removal of the old solution before more was added. The jars were brought to a specific weight before and after the renewal of the nutrient solution; hence the moisture content was uniformly maintained.

The oats were planted on December 20, 1926, and on January 28, 1927, when the more vigorous plants could be differentiated from the weaker ones, they were thinned to 18 plants per jar. On February 9 the first plants were harvested, nine being taken from each jar; on March 10 a second harvest was made; and on April 12 the remaining plants were taken up. Potassium and carbohydrate analyses were made on plants collected on all three dates. The results are presented in Table 1, A. The potassium was applied as follows: To jars 51 to 53, none; 56 to 58, 1 p. p. m.; 59, 61, and 62, 3 p. p. m.; 63, 65, and 66, 5 p. p. m.; and 68 to 70, 10 p. p. m.

The soybeans in sand cultures were treated in exactly the same manner as the oats. The seed was inoculated and planted on February 2, 1927, and after emergence the plants were thinned to six per jar. The first plant samples were taken on March 22 and the second on April 12, 1927. The potassium was applied as follows: To jars 131 and 132, none; 135 and 136, 1 p. p. m.; 139 and 140, 3 p. p. m.; 143 and 144, 5 p. p. m.; and 147 and 148, 10 p. p. m. These data are given in Table 5, A.

A series of soybeans (jars 149 to 154, Table 5, B) and a series of oats (jars 71 to 76, Table 1, B) were grown in sand through which the nutrient solution was allowed to percolate continuously. This was arranged by means of siphons, and the flow was controlled by varying the size of the channel outlet of rubber tubing by means of a Hoffman clamp.

TABLE 1.—*Variations in the carbohydrate constituents, dry matter, and potassium content of oat plants grown in sand cultures moistened with a balanced nutrient solution containing different amounts of potassium*

A. GROWN IN SAND CULTURE WITH NUTRIENT SOLUTION APPLIED DAILY

Jar No.	Date harvested	Potassium in solution	Plants per jar	Dry matter	Dry weight per 10 plants	Total sugars	Dextrins	Starch	Dextrins and starch	Sugars, dextrins, and starch	Hemicellulose	Potassium	Sugars, dextrins, and starch per plant
		P.p.m.	*No.*	*P. ct.*	*Gm.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*Mgm.*
53	Feb. 9	0	45	------	0.48	8.72	------	------	3.38	12.10	8.54	------	5.86
56	do	1	45	11.1	.57	10.33	------	------	1.48	11.81	9.21	0.64	6.83
61	do	3	45	11.1	.75	8.21	------	------	2.89	11.10	10.12	.83	8.32
63	do	5	45	10.8	.90	6.14	------	------	2.04	8.18	8.49	1.38	7.36
68	do	10	45	10.9	1.07	5.41	------	------	2.87	8.28	8.25	1.73	8.85
52	Mar. 10	0	9	27.0	1.4	8.00	------	------	2.12	10.12	9.70	------	14.16
57	do	1	9	21.6	3.5	10.04	------	------	1.40	11.44	10.87	.43	41.32
62	do	3	9	20.3	8.4	10.18	------	------	1.60	11.78	10.80	.60	98.95
66	do	5	9	19.1	10.7	11.52	------	------	2.15	13.67	10.05	.71	146.26
69	do	10	9	17.2	12.4	10.01	------	------	2.87	12.87	10.13	1.12	159.58
51	Apr. 12	0	9	------	1.7	------	------	------	------	------	------	------	------
58	do	1	9	------	------	------	------	------	------	------	------	.49	------
59	do	3	9	24.1	16.8	4.18	------	------	2.07	6.25	13.15	.39	105.00
65	do	5	9	22.0	27.5	3.89	------	------	2.86	6.75	14.72	.63	185.62
70	do	10	9	22.5	45.8	4.49	------	------	4.67	9.16	15.34	.85	419.52

B. GROWN IN SAND CULTURE WITH CONTINUOUS PERCOLATION OF NUTRIENT SOLUTION

71	Feb. 9	1	18	------	1.06	10.13	0	3.86	3.86	13.99	8.56	0.63	14.82
74	do	3	18	------	1.40	13.53	.43	4.97	5.40	18.93	9.18	.93	26.50
76	do	5	18	------	1.60	8.75	.16	1.89	2.05	10.80	9.17	1.10	17.28
72	Feb. 10	1	18	------	1.53	9.76	1.30	1.09	2.39	12.15	7.83	.93	18.58
73	do	3	18	------	2.00	13.14	.37	1.12	1.49	14.63	7.81	.99	29.30
75	do	5	18	------	1.76	8.65	.17	1.36	1.53	10.18	7.32	1.10	17.91

To maintain a uniform potassium concentration in the nutrient solution it was necessary to use water cultures. Soybeans and oats did not grow well in water cultures containing the above culture solution; therefore a modification of a solution devised by Hoagland and Martin (*14*) and Parker (*25*) was utilized. This solution was as follows: Solution A: 807 gm. Ca $(No_3)_2.4H_2O$; 143 gm. Mg $(NO_3)_2.6H_2O$; 5 gm. $CaCl_2$; 4 gm. $MgCl_2$; 1 gm. $H_3.BO_3$ (boric acid); water to make 2 liters. Solution B: 300 gm. $MgSO_4.7H_2O$; 1.2 gm. $MnSO_4$; water to make 2 liters. Solution C: 7 gm. $CaH_4(PO_4)_2.H_2O$; water to make 2 liters.

It was necessary to keep these solutions in separate containers in order to prevent the precipitation of some of the salts. Ten cubic centimeters of each of the above solutions were added to 7.5 liters of distilled water, the approximate amount necessary to fill one 2-gallon jar. The potassium was added as needed from either one of two stock solutions of potassium chloride. One solution contained 28.62 gm. of pure KCl per 2 liters, and 1 c. c. diluted to 7.5 liters was equivalent to 1 mgm. potassium per liter or 1.0 p. p. m.; the other contained 2.862 gm. KCl per 2 liters, and 1 c. c. when diluted to 7.5 liters was equivalent to 0.1 mgm. potassium per liter, or 0.1 p. p. m.

To maintain the amount of potassium in the solution as nearly constant as possible, it was renewed twice daily. To accomplish this, the following procedure was adopted: Two jars were filled with 7.5 liters of distilled water, and the required amount of the nutrient

solution, excluding potassium was added. The plants from the old potassium-free jar were then placed in the new potassium-free jar just renewed. This could easily be accomplished, because the plants were growing through holes in the wooden jar covers. (Fig. 3.) The old potassium-free jar was then made to 0.5 p. p. m. by the addition of the necessary amount of stock KCl solution. The plants were then transferred to this new 0.5 p. p. m. of potassium solution. To the old 0.5 p. p. m. jar an additional 0.5 p. p. m. of potassium was added, and it then became the 1 p. p. m. jar. Similarly, to the old 1 p. p. m. solution an additional 1 p. p. m. of potassium was added, and it became the 2 p. p. m. solution. This procedure was used for all jars. The solution in each 5.0 p. p. m. jar was discarded twice daily.

In order to keep the iron in solution, the jar containing the potassium-free solution was made to pH 4 every third day by the addition of normal HCl. Fifty cubic centimeters of a 0.5 per cent solution of ferric tartrate was then added. The plants were rotated so as to keep them feeding on the specified parts per million of potassium, and in the process of rotation all passed through the jars containing the iron every third day. No plant remained in the solution of pH 4 longer than 12 hours.

The plants grown in the water-culture solution were soybeans, cowpeas, cotton, and Sudan grass. These data are given in Tables 3, A and C, 4, A, and 5, C.

TABLE 2.—*Variations in the carbohydrate constituents, dry matter, and potassium content of oat and sweetclover plants when grown on Clarksville silt loam fertilized with different amounts of potassium*

A. OATS GROWN ON FIELD SOIL

Fertilizer	Sampling date	Plants	Dry matter	Dry weight per 10 plants	Reducing sugars	Total sugars	Dextrins	Starch	Dextrins and starch	Hemicellulose	Potassium	Sugars, dextrins, and starch	Sugars, dextrins, and starch per plant
	1927	*No.*	*P. ct.*	*Gm.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*Mgm.*
Check	June 30	50	37.30	22.69	0.152	0.220	1.287	0.963	2.250	15.95	2.69	2.470	56.01
NP	do	50	36.45	11.86	.152	.185	1.518	.750	2.268	17.15	2.79	2.453	29.09
NP K 150	do	50	31.30	17.53	.083	.152	.913	.663	1.576	18.60	3.93	1.728	30.29
NP K 300	do	50	28.62	19.45	.152	.152	1.000	.825	1.825	16.25	1.16	1.977	38.45
NP K 450	do	50	31.30	22.09	.152	.220	1.181	.775	1.956	15.05	2.98	2.176	48.06

B. OATS GROWN ON FIELD SOIL TAKEN TO THE GREENHOUSE

Check		8		3.39		7.75			3.70	8.97	2.27	11.45	38.64
NP		8		8.65		7.38			3.66	8.70	4.05	11.04	95.49
NP K 100		8		9.17		5.26			5.74	8.73	5.20	11.00	100.87
NP K 150		8		9.00		5.42			2.05	10.57	5.55	7.48	67.32

C. HUBAM SWEETCLOVER GROWN ON FIELD SOIL

	1927												
Check	July 8	40	23.22	13.20	3.86	4.36			7.15	12.38	2.12	11.51	152.0
NP	do	40	24.30	13.00	3.26	4.70			5.77	12.08	2.12	10.47	136.4
NP 150	do	40	29.00	18.50	3.04	4.95			5.49	12.26	1.92	10.44	193.1
NP 300	do	40	24.42	22.00	2.59	4.15			6.46	11.88	1.61	10.61	233.5
NP 450	do	40	21.05	16.50	3.82	5.30			5.33	12.00	1.70	10.63	175.5
	1928												
Check	July 31	100	29.0	15.90	1.187	2.475	1.794	1.406	3.200	13.620	1.75	5.675	90.23
NP	do	100	31.5	11.15	1.900	3.437	1.738	1.487	3.225	12.087	1.02	6.662	74.28
NP 150	do	100	31.5	13.86	2.187	3.580	2.000	1.963	3.963	10.275	1.57	7.543	104.54
NP 300	do	100	28.5	10.37	1.500	2.850	1.963	1.906	3.869	10.562	1.57	6.719	69.70
NP 450	do	100	30.0	15.00	1.625	3.250	1.888	1.666	3.554	13.470	2.30	6.804	102.06

TABLE 3.—*Variations in carbohydrate constituents, dry matter, and potassium content of cowpeas and cotton plants grown on field soil and in nutrient solutions containing different amounts of potassium*

A. COWPEAS GROWN IN WATER CULTURE

Potassium in solution and fertilizer	Sampling date	Plants	Dry matter	Dry weight per 10 plants	Reducing sugars	Total sugars	Dextrins	Starch	Dextrins and starch	Sugars, dextrins, and starch	Hemicellulose	Potassium	Sugars, dextrins, and starch per plant
P. p. m.:		*No.*	*P. ct.*	*Gm.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*Mgm.*
0		8	14.4	6.92	5.103	6.943	1.280	4.629	5.909	12.852	8.656	0.20	88.9
0.5		8	12.9	24.90	7.788	11.004	1.277	2.700	3.977	14.981	9.033	.71	373.0
1		8	12.6	38.20	9.147	12.566	1.051	2.239	3.290	15.856	7.992	.81	605.4
2		8	11.1	44.00	9.267	12.450	1.165	2.449	3.614	16.064	7.484	1.08	706.8
3		8	11.8	41.00	8.522	11.853	1.116	2.669	3.785	15.638	6.504	1.20	640.8
5		8	12.3	54.00	8.017	10.354	1.266	4.541	5.807	16.161	7.989	1.34	872.6

B. COWPEAS GROWN ON FIELD SOIL

	1928												
Check	Aug. 16	59	15.25	94.6	1.087	2.975	1.831	3.150	4.981	7.956	7.412	2.25	754.00
NP	do	59	14.75	96.6	1.647	3.775	1.888	3.031	4.919	8.694	7.370	2.29	839.84
NP K 150	do	59	14.00	87.6	1.647	3.700	2.112	3.218	5.330	9.030	5.150	2.95	791.62
NP K 300	do	59	14.25	87.6	.995	2.850	1.950	3.162	5.112	7.962	7.000	3.00	697.47
NP K 450	do	59	13.00	91.2	.760	2.470	1.920	3.837	5.757	8.227	4.587	3.38	750.30
Check	Aug. 31	39	16.00	36.8	1.225	3.439	2.037	6.306	8.343	11.772	6.090	2.13	433.21
NP	do	39	15.00	43.6	1.362	3.625	1.325	4.775	6.100	9.725	6.625	2.26	424.61
NP K 150	do	39	15.00	37.0	1.875	4.000	2.331	5.625	7.956	11.956	6.550	2.33	442.37
NP K 300	do	39	15.00	41.0	1.225	3.075	1.218	5.462	6.680	9.755	5.420	2.32	399.95
NP K 450	do	39	14.50	42.5	.900	2.709	1.350	5.390	6.650	9.350	6.850	3.02	391.60

C. COTTON GROWN IN WATER CULTURE

P. p. m.:													
0		8	20.9	5.7	2.100	2.660	0.880	2.290	3.170	5.830	7.450	0.78	33.23
0.5		8	19.7	10.0	2.616	3.133	.966	2.170	3.136	6.269	9.441	.81	62.69
1		8	19.0	11.6	2.768	3.087	1.260	3.218	4.478	7.565	10.625	1.18	82.94
2		8	18.3	15.5	2.900	3.800	1.325	4.387	5.712	9.512	11.000	1.10	147.83
3		8	17.1	16.0	2.375	2.900	1.550	3.250	4.800	7.700	10.700	1.48	123.20
5		8	17.3	16.6	2.775	3.750	1.033	2.750	3.783	7.533	10.050	1.80	124.50

The crops used in field study were soybeans, cowpeas, oats, corn, sweetclover, and Sudan grass. These crops were grown in quadruplicate. In some instances samples for analysis were collected from all four plots at different stages of plant growth. The series of treatments given are: No treatment (check); nitrogen and phosphorus only; nitrogen and phosphorus with 150 pounds KCl; nitrogen and phosphorus with 300 pounds KCl; and nitrogen and phosphorus with 450 pounds KCl to the acre. The results are given in Tables 2, A, B, C; 3, B; 4, B, C; and 6, A, B.

All plant tissue obtained from the cultures-solution work used for carbohydrate analyses was preserved in alcohol which was boiling when added. The field samples were dried after the method described by Link (*20*). The method of carbohydrate analysis was essentially the same as that of Murneek (*24*) and Janssen (*16*).

In discussing the relation of carbohydrate production to the percentage of potassium in the plant, emphasis is placed on the total percentage of sugars, dextrins, and starch. These data are given as a separate column in the tables. It was thought that this method of comparison would give a better index of the effect of potassium on the

carbohydrate compounds in the plant than if each compound were discussed separately. Similarly, the term "total carbohydrate compounds" includes the total weight of sugars, dextrins, and starch per plant. The percentages of hemicellulose have not been included with the sum of the percentages of sugars and starch because hemicelluloses are of a more complex nature. The data for hemicellulose are listed, but will not be discussed, since they are so variable.

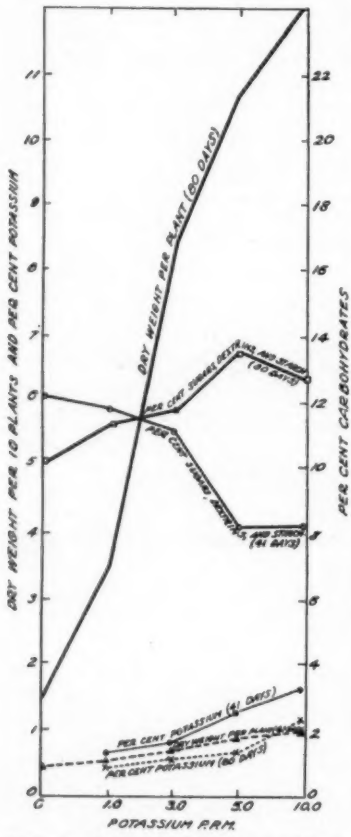

FIGURE 1.—Relation between the dry weight, potassium content, and carbohydrate constituents of oats when grown in a nutrient solution containing varying amounts of potassium

RESULTS

The data for carbohydrate and potassium analyses of plants grown in sand cultures are given in Table 1. The results of the analyses for the February 9 and March 10 collections are presented also in Figure 1, A. Large differences in growth were observed in plants receiving the various potassium treatments. (Fig. 2.) The dry weights in Table 1 show decided differences in favor of the heavier treatments of 5 and 10 p. p. m.

Table 1, B, gives data obtained from plants grown in sand but supplied with a nutrient solution which was allowed to percolate continuously on the sand in the jar. This table indicates that the maximum growth based on dry weight was obtained when the plants received the 3 p. p. m. potassium treatment. The percentage of total sugars and starch seems to bear this out.

Table 2, A and B, shows the effect of various amounts of potassium on soil cropped to oats. The soil used was classified as Clarksville silt loam. The data here shown have little significance when considered from the standpoint of the effect of potash fertilization on the growth of the plants. Neither the sugar nor the starch shows any constant relationship to the amounts of potassium added to the soil, except that there is a slight decrease in the percentage of these compounds with the increments of potassium. It should be stated that the oat plants taken from the field were stunted by excessive summer heat and were beginning to dry up when the samples were collected.

Carbohydrate analyses were not made on sweetclover plants grown in culture solution, but the results of analyses of plants grown in field soil to which KCl was added are given in Table 2, C. Analyses were

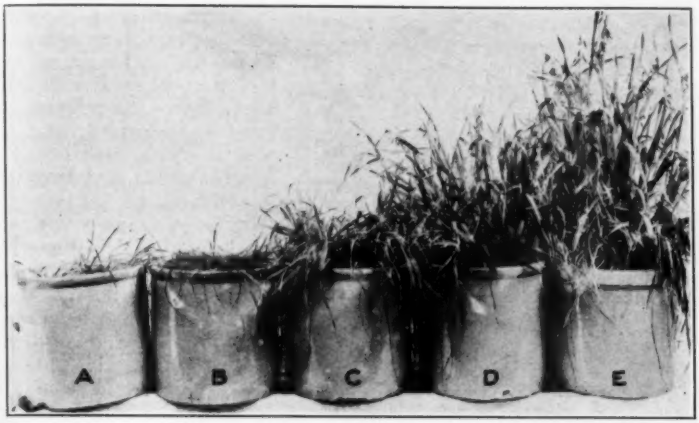

FIGURE 2.—Fulghum oats grown on sand cultures containing the following amounts of potassium in parts per million: A, 0; B, 1; C, 3; D, 5; E, 10

made of plants collected in 1927 and 1928. The data for 1927 show the greatest dry weight per plant obtained on soil which received 300 pounds of KCl. There are no noticeable differences in the percentage of sugars and starch in plants containing different percentages of potassium. The results for 1928 show that there was little difference

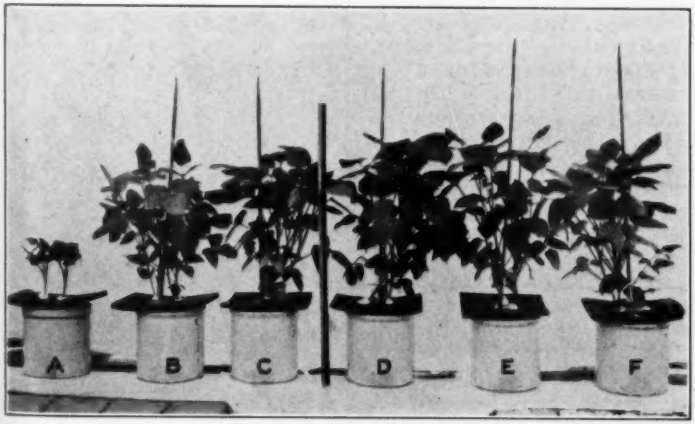

FIGURE 3.—Cowpeas grown in nutrient solutions containing the following amounts of potassium in parts per million: A, 0; B, 0.5; C, 1; D, 2; E, 3; F, 5

in total plant growth resulting from potash fertilization, but that the percentage of sugars and starch increased slightly in plants grown on potash-fertilized soil.

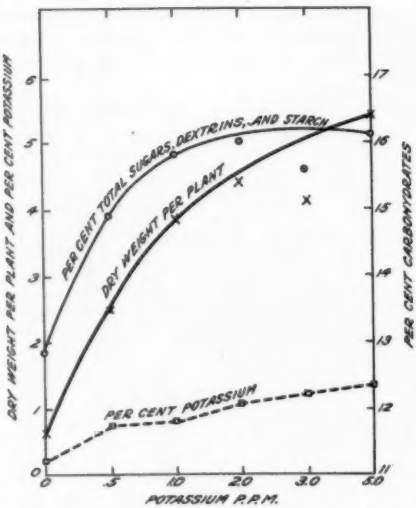
DRY WEIGHT PER PLANT AND PER CENT POTASSIUM
PER CENT CARBOHYDRATES
PER CENT TOTAL SUGARS, DEXTRINS, AND STARCH
DRY WEIGHT PER PLANT
PER CENT POTASSIUM
POTASSIUM P.P.M.

FIGURE 4.—Relations between the dry weight, potassium content, and carbohydrate constituents of cowpea plants when grown in nutrient solutions containing different amounts of potassium

The data for cowpeas grown in water-culture solutions are presented in Table 3, A. These plants made excellent growth in water cultures and were harvested 38 days after they were first placed in the solutions. Figure 3 shows the relative differences in growth of these plants. The greatest dry weight per plant was obtained from plants grown in solutions receiving 2, 3, and 5 p. p. m. of potassium. The .5 p. p. m. plants were allowed to grow 48 hours longer than the others (in order that a study might be made of the rate of potassium uptake), and for that reason the dry weight was greater than the 2 and 3 p. p. m. plants.

The data for these plants are presented graphically in Figure 4. This figure indicates that the weight per plant, as well as the percentage of sugar and starch, increases with the increment of potassium in the plant. However, the ratio of the percentages of sugars and starch to the percentage of potassium in the plant decreased at the higher concentrations of potassium. This is evidenced by the graph showing percentage sugars and starch at the higher potassium concentrations.

Table 3, B, gives the results of the analysis of cowpea plants grown on field soil fertilized with potassium. The plants were harvested at two different stages of development, as indicated. No relation is shown between the dry weight per plant and the amounts of

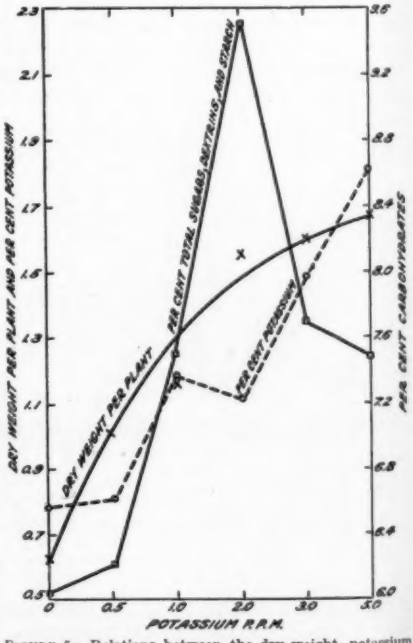
DRY WEIGHT PER PLANT AND PER CENT POTASSIUM
PER CENT CARBOHYDRATES
PER CENT TOTAL SUGARS, DEXTRINS, AND STARCH
DRY WEIGHT PER PLANT
PER CENT POTASSIUM
POTASSIUM P.P.M.

FIGURE 5.—Relations between the dry weight, potassium content, and carbohydrate constituents of cotton plants when grown in nutrient solutions containing different amounts of potassium

potassium added to the soil. Among the samples collected on August 16, 1928, the potassium-fertilized plants show a small increase in percentage of dextrins and starch, but no such increase is noted in the August 31 collection. The total sugars and starch varied in plants receiving different quantities of potassium. However, there are fairly consistent increments in the percentage of potassium in the plants with the higher rates of potassium fertilization.

Table 3, C, and Figure 5 give the data for cotton (Trice 323) grown in water cultures and harvested 81 days after planting. The dry weight per plant increased with the increments of potassium in the nutrient solution, but the sum of the percentages of sugars, dextrins, and starch decreased at 3 and 5 p. p. m. of potassium. The highest percentages of sugars, starch, and dextrins were obtained in plants grown on nutrient solutions containing 2 p. p. m. of potassium.

TABLE 4.—*Percentage carbohydrate constituents, dry matter, and potassium in corn and Sudan grass grown in soil or in nutrient solutions containing different amounts of potassium*

A. SUDAN GRASS GROWN WATER CULTURE

Potassium in solution or fertilizer	Part of plant	Plants	Dry matter	Dry weight per 10 plants	Reducing sugars	Total sugars	Dextrins	Starch	Dextrins and starch	Sugars, dextrins, and starch	Hemicelluloses	Potassium	Sugars, dextrins, and starch per plant
P. p. m.:		*No.*	*P. ct.*	*Gm.*	*P. ct.*	*P. ct.*	*Pc. t.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct*	*P. ct.*	*Mgm.*
0		12	22.4	4.42	5.558	8.909	0.828	1.432	2.260	11.169	16.085	0.22	49.36
0.5		12	20.9	37.60	7.710	13.530	.658	1.987	2.645	16.175	18.910	.52	608.18
1.0		12	20.4	44.50	9.490	14.820	.758	1.383	2.140	16.960	16.110	.68	751.76
2.0		12	19.3	36.00	9.210	16.010	.680	2.249	2.929	18.939	18.320	.92	681.73
3.0		12	18.1	59.20	7.583	11.366	1.254	2.187	3.441	14.807	13.590	.84	876.57
5.0		12	18.0	60.00	6.078	11.506	1.242	1.885	3.127	14.633	17.225	1.07	877.98

B. SUDAN GRASS GROWN ON FIELD SOIL

Check		[a] 40	20.7	8.87	5.34	8.35	.....	2.70	.....	11.05	15.7	1.60	98.010
NP		40	21.3	14.60	6.10	10.58	.....	2.97	.....	13.55	13.2	1.36	197.830
NP K 150 [b]		40	20.3	10.50	5.25	10.49	.....	3.58	.....	14.07	14.1	1.95	147.735
NP K 300		40	19.2	9.37	6.18	9.41	.....	2.27	.....	11.68	13.6	2.02	109.675
NP K 450		40	19.2	8.01	4.00	7.94	.....	2.90	.....	10.84	13.4	1.98	86.820

C. CORN GROWN ON FIELD SOIL

Check	Stalk	3	15.0	497.5	13.025	17.337	1.830	1.943	3.773	21.110	13.775	2.92	[c] 10.500
NP	...do...	3	15.0	339.0	11.750	16.825	2.018	1.587	3.606	20.431	13.587	2.12	6.835
NP K 150	...do...	3	15.0	655.0	9.712	14.287	2.825	1.037	3.862	18.149	13.325	3.70	11.887
NP K 300	...do...	3	15.0	775.0	9.375	12.875	2.731	1.681	4.412	17.287	13.437	4.44	13.397
NP K 450	...do...	3	16.0	711.4	6.250	8.862	1.737	2.131	3.868	12.730	13.475	5.03	9.056
Check	Leaves	3	22.5	409.0	1.075	2.662	1.080	1.606	2.686	5.348	14.312	2.22	2.180
NP	...do...	3	14.0	225.4	3.325	5.425	.700	2.469	3.169	8.594	14.612	1.78	1.937
NP K 150	...do...	3	18.7	387.0	2.662	3.737	.484	1.812	2.296	6.033	13.775	2.51	2.334
NP K 300	...do...	3	18.0	432.0	1.100	2.862	.712	2.219	2.931	5.793	13.100	2.84	2.503
NP K 450	...do...	3	16.0	368.0	1.700	3.662	.471	4.063	4.534	8.196	14.275	2.36	3.016

[a] Roots removed.
[b] Pounds per acre of KCl in sections B and C.
[c] Unit is grams in this column of section C.

The results of the analyses of Sudan grass grown in water-culture solutions are presented in Table 4, A, and in Figure 6. These data indicate that up to a certain amount, 3 p. p. m., the dry weight per plant tends to increase with the percentage of potassium in the nutrient solution. They also show, as in the case of cotton (fig. 4), that

the percentages of sugars, dextrins, and starch is greatest in plants when grown in nutrient solutions containing 2 p. p. m. of potassium.

These results obtained on plants grown in culture solutions seem to be confirmed by those obtained from analyses of Sudan grass plants taken from field soils treated with various amounts of potassium, as shown in Table 4, B. It will be noted, first, that the total dry weight per plant bears no direct relation to the amount of potassium applied to the soil. However, the final field hay weights show the following results: No potassium, 73.4 pounds; 150 pounds KCl, 85.8; 300 pounds KCl, 91.1; and 450 pounds KCl, 91.1. These results indicate that maximum plant development was reached when potassium was applied at the rate of 300 pounds KCl per acre. The highest percentage of sugars and starch was obtained when the plants were grown on soil which received a 150-pound application of KCl.

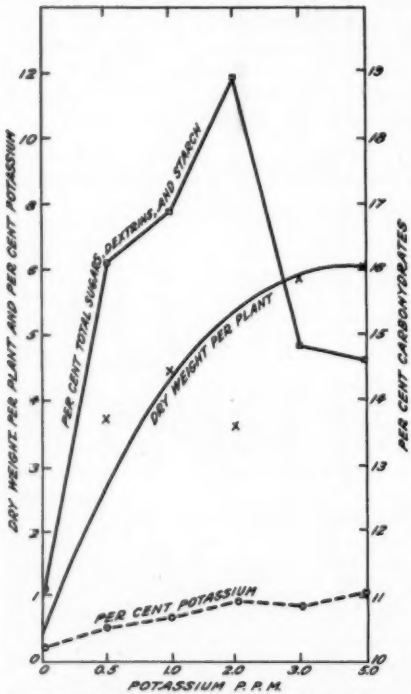

FIGURE 6.—Relations between the dry weight, potassium content, and carbohydrate constituents of Sudan grass when grown in nutrient solutions containing different amounts of potassium

Corn was not grown in either water or sand cultures, but plants were collected from a field fertilized with KCl. The plant samplings were made a short time before tasseling, and the leaves and stalks were analyzed separately. The results are presented in Table 4, C. The stalks gradually decreased in total sugars and starch with increase of potassium in the plant. In the leaves there is noted a fluctuation in percentage of sugar and starch, but the percentage of these compounds in plants grown on soils receiving a complete fertilizer was no greater than in those receiving only nitrogen and phosphorus.

Data on soybean plants grown in sand-culture solutions are given in Table 5, A and B. The data shown in Table 5, A, are also presented in Figure 7. At both stages of plant development there is an increase in dry weight accompanying the increase of potassium in the nutrient solution upon which the plants were grown.

The sum of percentages of sugars and starch decreased in plants of the first collection with increase of potassium but increased in the second collection. This reverse change, like that in the case of oats (Table 1, A), may perhaps be accounted for by the presence of residual

potassium in the seed which supplied the plant in early development, whereas in later growth the potassium deficiency became pronounced, resulting in decreased carbohydrate synthesis.

TABLE 5.—*Variations in carbohydrate constituents, dry matter, and potassium content of soybean plants grown on a balanced nutrient solution containing different amounts of potassium*

A. GROWN IN SAND CULTURE

Jar No.	Date harvested	Potassium in solution	Plants per jar	Dry matter	Dry weight per 10 plants	Reducing sugars	Total sugars	Dextrins	Starch	Dextrins and starch	Sugars, dextrins, and starch	Hemicellulose	Potassium	Sugars, dextrins, and starch per plant
		P. p. m.	*No.*	*P. ct.*	*Gm.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*Mgm.*
132[a]	Mar. 22	0	6											
135	do	1	6		4.3		2.35		4.43		6.78	6.66	0.78	29.15
139	do	3	6		7.9		1.96		3.28		5.24	8.63	.81	41.39
143	do	5	6		9.6		2.43		2.84		5.27	8.20	1.00	50.59
147	do	10	6		13.0		2.79		2.11		4.90	8.96	.86	63.70
131	Apr. 12	0	6		4.8		1.50		.82		2.32	8.70	.40	11.13
136	do	1	6		10.8		1.70		1.18		2.88	8.06	.42	31.10
140	do	3	6		18.0		1.02		1.60		2.62	7.62	.62	47.16
144	do	5	6		26.0		1.30		3.41		4.71	8.61	.68	122.46
148	do	10	6		31.6		1.48		6.23		7.71	7.4	.86	243.63

B. GROWN IN SAND CULTURE WITH CONTINUOUS PERCOLATION OF NUTRIENT SOLUTION

149–50		1	6		7.1		2.75		2.22		4.97	8.14	.80	35.28
151–52		3	6		11.4		2.53		3.29		5.82	8.05	.78	66.40
153–54		5	6		12.9		2.61		3.60		6.21	8.53	.85	80.10

C. GROWN IN WATER CULTURE

		0	8	20.83	4.7	1.653	2.480	0.799	2.148	2.947	5.427	11.119		25.4
		0.5	8	15.90	14.8	1.210	1.772	.778	1.070	1.848	3.620	10.740	1.13	53.4
		1.0	8	15.00	13.0	1.040	1.489	.893	1.117	2.010	3.499	10.969	1.63	45.7
		2.0	8	14.55	17.6	.932	1.526	.974	1.185	2.159	3.685	11.792	1.77	64.9
		3.0	8	14.05	17.0	.852	1.476	1.061	.883	1.944	3.420	10.232	2.21	58.2
		5.0	8	14.70	19.3	.836	1.426	.762	1.059	1.821	3.247	11.113	1.97	62.6

[a] Sample was lost.

The data in Table 5, B, for soybeans grown in sand to which nutrient solutions were added by continuous percolation show the greatest dry weight per plant and the highest percentage of sugar and starch in the plants that received a nutrient solution containing 5 p. p. m. potassium.

The data for soybeans grown in water-culture solutions to which various increments of potassium were added are given in Table 5, C. The trend of the total dry weight per plant up to a concentration of 2 p. p. m. of potassium was to increase with the increase in percentage of potassium in the nutrient solution. Though good total growth was obtained, the plants were abnormally long and somewhat viney. The percentage of sugars and starch are more or less similar in plants grown at all potassium concentrations. Only those plants receiving no potassium showed a decided increase in these compounds. This can not be accounted for.

TABLE 6.—*Variations in the carbohydrate constituents, dry matter, and potassium content of soybean plants when grown on Clarksville silt loam fertilized with different amounts of potassium*

A. SOYBEANS GROWN ON FIELD SOIL IN 1927

Fertilizer	Sampling date	Part of plant	Plants	Dry matter	Dry weight of 10 plants	Reducing sugars	Total sugars	Dextrins	Starch	Dextrines and starch	Sugars, dextrins, and starch	Hemicellulose	Potassium	Sugars, dextrins, and starch in plants
			No.	*P. ct.*	*Gm.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*Mgm.*
Check	June 30	Stem	50	20.67	7.01	2.21	3.90	1.42	3.72	5.149	9.049	11.79	2.19	63.28
NP	do	do	50	22.00	10.20	2.44	3.82	1.35	3.00	4.352	8.172	12.72	1.77	83.35
NP K 150	do	do	50	19.32	10.73	1.74	3.04	1.42	3.22	4.649	7.689	13.33	2.24	82.50
NP K 300	do	do	50	18.00	12.70									
NP K 450	do	do	50	19.32	16.75	1.36	2.74	1.23	1.59	2.927	5.667	13.66	2.44	94.92
Check	do	Leaf	50	25.79	5.00	.82	3.19	2.59	5.61	8.20	11.290	10.80	1.93	56.50
NP	do	do	50	22.75	6.58	.75	1.98	2.40	5.37	7.774	9.754	11.54	1.64	64.18
NP K 150	do	do	50	22.74	7.58	.97	2.05	2.51	6.04	8.555	10.605	11.40	1.97	80.38
NP K 300	do	do	50	22.00	10.07	.82	2.21	2.55	5.64	8.190	10.400	12.12	1.84	104.80
NP K 450	do	do	50	20.67	11.37	.89	2.29	2.59	4.01	6.600	8.890	10.54	2.00	102.21

B. SOYBEANS GROWN ON FIELD SOIL IN 1928

Check	Aug. 16	Entire	100	20.75	16.081	1.699	2.616	1.45	4.391	5.849	8.465	10.03	1.38	136.11
NP	do	do	100	20.00	16.520	1.749	2.533	1.68	5.041	6.724	9.257	10.73	1.65	152.92
NP K 150	do	do	100	19.00	16.796	1.899	2.816	1.62	5.516	7.141	9.957	10.43	1.97	167.17
NP K 300	do	do	100	19.75	20.145	1.326	2.199	1.40	5.516	6.924	9.123	10.43	2.34	183.78
NP K 450	do	do	100	19.25	17.864	1.396	2.533	1.19	4.991	6.190	8.723	10.93	2.07	155.79
Check	Sept. 1	do	40	20.5	11.00	1.53	2.61	1.35	5.116	6.466	9.076	12.16	1.56	249.59
NP	do	do	40	21.0	17.43	1.53	2.56	1.57	4.716	6.286	8.846	12.21	1.27	385.42
NP K 150	do	do	40	21.0	19.40	1.53	2.34	1.48	5.336	6.916	9.156	13.26	1.68	444.06
NP K 300	do	do	40	21.0	22.26	1.55	2.62	1.31	4.506	5.816	8.436	12.32	1.54	469.04
NP K 450	do	do	40	21.0	23.47	1.362	2.40	1.51	5.663	7.173	9.573	11.50	1.87	561.93

In 1927 samples of soybeans were collected from field plots fertilized with various amounts of potassium. The plants were separated into stem and leaves and analyzed. The data are given in Table 6, A. The plants grown on plots receiving the largest potassium application (450 pounds KCl per acre) had the largest total dry weight. The percentage of sugars and starch in the stems of these plants decreased with an increase in the amount of potassium added to the soil. These results are similar to those obtained from cornstalks. (Table 4, C.) The leaves of the soybeans from the various potassium soil treatments show practically no difference in the combined percentage of sugars and starch.

The analyses of soybeans collected in 1928 at two stages of development (Table 6, B) also show with the exception of the August 16 sample from the heaviest potassium application an increase in dry weight per plant with the increase in potassium fertilizer applied to the soil. In the first collection the total percentage of sugars and starch is slightly higher in plants grown on plots receiving the 150 pounds per acre KCl application; for the second collection, September 1, 1928, the results fluctuate and no definite relationship appears to exist.

The data for the sum of the total sugars, dextrins, and starch per plant of oats and soybeans grown in sand and for cotton, Sudan grass, and cowpeas grown in water are shown in milligrams in Figure 8. This figure shows that in so far as the total amounts of these substances

are concerned they are directly correlated with the highest percentage of potassium in the nutrient solution on which the plants grew.

DISCUSSION

The results of an earlier (*2*) investigation of plants grown in water cultures with potassium concentrations ranging from 1 to 5 p. p. m. showed that the largest percentage of potassium was taken up by the plants grown on the medium receiving the heaviest application of potassium. These results agree with those of other workers (*5*, *6*, *7*, *8*, *9*). The data further indicated that more potassium was taken from nutrient solutions containing large amounts of this ion than was actually required for the needs of the plant. Similar results were obtained in the present experiments, as shown by the total dry weights and percentages of potassium in plants. (Tables 1, B; 3, A; 4, A; and 5, C.) All these plants were grown in solutions in which the parts per million of potassium were kept as nearly constant as possible. Similar relations, however, of total dry weight to percentage of potassium in the plant under field soil conditions will be noted in Tables 2, A and C; 3, B and C; and 6, B.

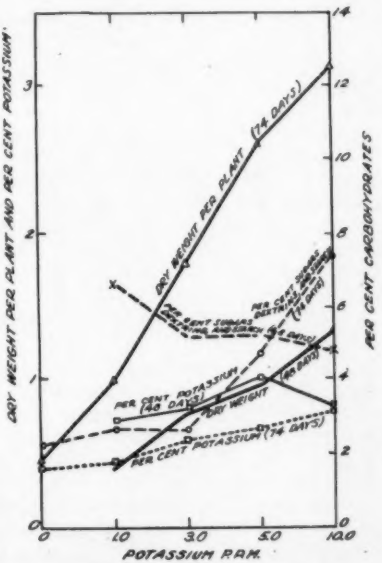

FIGURE 7.—Relations between the dry weight, potassium content, and carbohydrate constituents of soybean plants when grown in sand cultures containing different amounts of potassium

This indicates that a greater percentage of potassium is taken up by the plant than is actually needed in its metabolism. These results have been noted in a previous article (*2*) and are in agreement with the findings of Hopkins (*15*), Lende (*19*), and Blair (*3*).

It will be noted from Tables 1 to 6 that in many instances there is no significant relationship between the potassium of a plant and the amount of sugars and starch present. Thus oats grown in sand cultures (Table 1, A, and fig. 1) to which increments of potassium were added, and harvested February 9, show no definite relation of total percentage of sugar and starch to the percentage of potassium in the plant. However, the data from analyses of plants harvested on March 10 and April 12 do show some significant relation in total percentage of sugars and starch to that of the higher percentages of potassium in the plant. This lack of correlation between sugars, starch, and potassium in the plants first harvested may have been due to the fact that the potassium in the seed had been sufficient to supply the plant to at least this stage of development. At the time of the earlier harvest the plants were approximately 6 inches high.

A similar relationship between the percentage of carbohydrates and the percentage of potassium in the plant may be noted in the case of soybeans. (Table 5, A, and fig. 7.) In this case, as in the oats, there is for the first analysis (March 22, 1927) a decrease up to 5 p. p. m. of potassium in the nutrient solution in the percentage of total sugars and starch with an increase in the percentage of potassium in the plant and for the second analyses (April 12, 1927) there is an increase. The same explanation given for the oats may hold true in the case of the soybeans. However, when the data for the oats (Table 1, B) and soybeans (Table 5, B) grown in sand, with continuous percolation of the nutrient solution, are examined it will be seen that the highest percentage of sugars and starch was obtained in the plants grown in nutrient solutions containing 3 p. p. m. of potassium, whereas in the case of soybeans there is a consistent increase in the carbohydrate compounds in plants up to 5 p. p. m. of potassium in the solution.

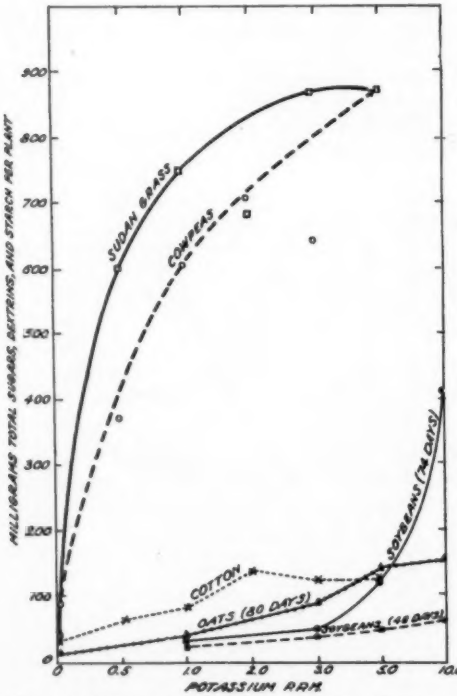

FIGURE 8.—Relations between the total sugars, dextrins, and starch of different plants when grown on nutrient solutions containing different amounts of potassium

Figures 4, 5, and 6, for cowpeas, cotton, and Sudan grass grown in water-culture solutions to which the increments of potassium added over the potassium-free solution were 0.5, 1.0, 2.0, 3.0, and 5.0 p. p. m. and kept at these concentrations by renewing the amounts twice daily, show that there is a tendency for the percentage of sugar and starch to decrease at about 2 to 3 p. p. m. of potassium. These results agree with the work of Stoklasa and Pitra (*30*), who grew barley in pot cultures with full fertilizers but with different quantities of potassium. They used the following amounts of KCl per jar: 0.5, 1.0, 1.5, and 3.0 gm. The percentage of starch in these plants is, respectively, as follows: 57.38, 63.52, 64.00, 61.20, and 57.68, showing that the greatest percentage of starch was obtained at about 0.5 and 1 gm. KCl per jar. These data indicate that when an active supply of potassium is present in the soil solution the plant takes up more than is actually necessary for its metabolism. It seems therefore, very probable that the greatest plant growth would not take

place at the maximum potassium concentration of the soil solution, but at some intermediate point. Similarly, the maximum percentages of sugars and starch do not necessarily occur in plants when the potassium concentration is greatest, as may be observed in these experiments, but rather at an intermediate point. In the work reported no plants were left growing to maturity, nor were any seeds analyzed, nor were any plants used in which special storage organs were concerned, such as roots or tubers. The nearest approach to such organs are the stems of the plants used.

Other investigators seem also to have found that there is not always a direct correlation between the potassium in the plant and sugar produced. Thus, Andrlik (*1*) has noted that the proportion of potassium taken up by the plant to the quantity of sugar produced was not constant, but varied from 2.1 to 5.8 parts potassium to 100 parts sugar. Schneidewind (*27*), in experiments with potatoes on sand and with the use of different fertilizers, showed that the use of a 40 per cent potassium salt as an application in the spring caused a marked reduction in the starch content of potatoes. However, with potassium sulphate the starch content was increased. In the chemical investigations of Morse and Jones, as reported by Haskell (*13*), it was found that sugars were consistently lower in the wood of plants grown on soil treated with potassium chloride. Kraft (*18*) noted that potassium fertilizers alone heavily decreased the dry matter, starch, and protein content of potatoes, but increased the percentage of water and mineral matter and had an unfavorable action on flavor.

These investigations suggest that the type of fertilizers, the type of plants, and the nature of the environment play a large part in the response of the plant to potassium. The effect of temperature on the assimilation of potassium in the plant has been shown by Tottingham (*32*), and his results indicate that temperature may be an important factor in potassium assimilation. It is very possible that the above factors would greatly modify the potassium and carbohydrate relationship.

In order to correlate the total sugars and starch per plant with parts per million of potassium in the nutrient solution upon which the plants were grown, the total weight of sugars and starch per plant of cowpeas, Sudan grass, cotton, soybeans, and oats have been graphed in Figure 8. With the exception of the cotton plant, which shows a greater amount of total sugar and starch per plant at 2 p. p. m. of potassium, all other plants named show a greater quantity of these compounds at the highest concentration of potassium used in the nutrient solution. If these data are compared with the percentage composition of these same compound figures it will be seen that the largest percentage of these compounds was obtained when the nutrient solution contained approximately 2 or 3 p. p. m. of potassium. The relationship of total sugars and starch compounds per plant between plants grown on soils fertilized with potash and plants grown on soils not fertilized with potash is in harmony with data presented by Hall (*11*) and Stoklasa (*29*). However, it should be expected that as the dry weight per plant increases, the total sugars and starch should also increase, even though the percentage of these compounds remained the same. Hence the total amount of these compounds in the plants can not be used as a criterion by which to estimate the activity of potassium in their formation. If, on the other hand, the

percentage of these compounds is considered with that of potassium it will be noted that the maximum increase takes place, not at the greatest percentage of potassium in the plant but at an intermediate point.

It appears, therefore, that there is an optimum potassium concentration in the plant at which CO^2 assimilation is at its maximum and above or below which it again decreases. These optimum relationships no doubt are difficult to define. The most that can be said is that they are as complex for the potassium ion as for any other ion in the plant, and, similarly, that the optimum under one set of environmental conditions may be different from that under other environmental conditions.

SUCCULENCE

In a previous paper (*17*) it was shown that tomato plants grown on a nutrient solution containing a large amount of potassium were more succulent than plants grown on a nutrient solution containing a very small amount of potassium. The degree of succulence was determined by the percentage of dry matter of the two types of plants.

In the present investigation attention is again called to the greater percentage of dry matter in the plants receiving small amounts of potassium. Thus oats grown on sand cultures which received no or little potassium, as compared with cultures which received 10 p. p. m. potassium, show the following differences in percentages of dry matter (Table 1, A) in favor of the plants grown on the no-potassium solution for the March 10 collections and on the 3 p. p. m. solution for the April 12 collection: 9.8 per cent and 1.6 per cent, respectively. Similarly, plants grown in water cultures with 0 or 5 p. p. m. of potassium show the following differences in dry matter: Cowpeas, 2.1 per cent (Table 3, A); cotton, 3.6 per cent (Table 3, C); Sudan grass, 4.4 per cent (Table 4, A); and soybeans, 6.13 per cent (Table 5, C) in favor of plants grown on a no-potassium solution.

The plants collected from the field soils to which various amounts of potassium were applied also show differences in percentage of dry weight. Thus plants grown on soil which received no potassium, as compared with those grown on soil which received muriate of potassium at the rate of 450 pounds per acre, show the following differences in percentage of dry matter: Oats, 6 per cent (Table 2, A); cowpeas, first collection, 2.25 per cent, second collection, 1.5 per cent (Table 3, B); Sudan grass, 1.5 per cent (Table 4, B); and corn leaves, 6.5 per cent (Table 4, C) in favor of plants grown on soil poor in potassium.

The data presented show quite conclusively that high-potassium plants are more succulent than low-potassium plants. Whether this is due to the better utilization of the nitrogen in the plant through the direct action of potassium or to the function of potassium in the formation of the precursory carbohydrate compounds, or even to the action of potassium alone, is a matter of conjecture.

SUMMARY

The present investigation includes the determination of potassium, sugars, dextrin, starch, and hemicellulose in soybeans, cowpeas, Sudan grass, oats, sweetclover, and corn grown in soil, sand, and

water culture solutions to which various amounts of potassium were added.

From the results of the work on water and sand culture as well as on field soils to which increments of potassium were added in amounts of 0 to 5 p. p. m. potassium for water culture, 0 to 10 p. p. m. potassium for sand culture, and 450 pounds KCl per acre on field soil, it is evident that plants vary with respect to the amount of potassium needed for the production of normal growth. It was found in all this work that the largest plant growth was obtained at an intermediate application of potassium, which in water cultures would appear to be about 2 to 3 p. p. m. of potassium as evidenced by dry weight per plant.

When the percentage of sugars and starch in plants grown in culture solution were totaled it was found that the largest percentage of these compounds was obtained at 2 to 3 p. p. m. of potassium, which was below the concentration at which maximum absorption of potassium took place. These results show that more potassium is actually taken up by the plant than is needed for its use.

The relation between the percentage of potash and that of carbohydrate compounds fluctuates greatly, and it appears that a high percentage of sugars and starch is not necessarily associated with a high percentage of potassium in the plant.

If the total weight of sugars and starch per plant is compared with the percentage of potassium in the plant, it becomes evident that the correlation is good.

Dry weight per plant decreases with the increase of potassium in the plant, or with the amount of potassium in the nutrient solution. The indications are that as the amount of potassium in the nutrient solution increases the succulence of the plant also increases.

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A STUDY OF THE MOSAIC DISEASE OF CRUCIFERS [1]

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INTRODUCTION

Mosaic diseases of plants have become increasingly serious during the last quarter of a century, or else the damage done by them has become more fully recognized. In the course of a general investigation of diseases of cruciferous crops the writer has given particular attention to the mosaic disease affecting them. The trouble was observed in rutabaga fields on Long Island, and, in the beginning, there was a question as to whether a disease of cauliflower called whiptail might not be a mosaic. The malnutritional nature of the whiptail disease has been established,[2] however, and it is intended here to record the results of the investigations of the true cruciferous mosaic.

Literature references to the cruciferous mosaic in this country are limited to simultaneous publications by Gardner and Kendrick [3] and Schultz [4] recording its appearance and the infectious nature of the disease. Schultz found the mosaic intertransmissible between turnip, mustard, and Chinese cabbage, but the potato and morning-glory were not infected. He also found that mustard seed from mosaic plants produced healthy seedlings. Gardner and Kendrick reported that the disease was easily transmitted from turnip to turnip but not from turnip to radish. Gram [5] states that in Denmark the disease was observed on turnips in a number of localities and that different varieties of turnips, rutabagas, Raphanus, and *Sinapsis arvensis* were susceptible.

The chief object of the investigation reported in this paper was to determine whether the mosaic disease known to attack certain crucifers was potentially a serious trouble of any of the three important cruciferous crops grown on Long Island, N. Y.; that is, cabbage, cauliflower, and Brussels sprouts.

RELATION OF DISEASE TO SEED

In the fall of 1923 the writer selected 20 rutabagas from a healthy field and a like number of mosaic-affected roots from a near-by field in which about 5 per cent of the plants were diseased. These two lots were stored separately and during mid-March of the following year 12 of the best roots from each lot were set out in the greenhouse. For various reasons 2 plants of each lot were later removed, but the remaining 18 plants grew and bore seed. It was observed

[1] Received for publication May 8, 1929; issued February, 1930.

[2] CLAYTON, E. E. INVESTIGATIONS OF CAULIFLOWER DISEASES ON LONG ISLAND. N. Y. State Agr. Expt. Sta. Bul. 506, 15 p., illus. 1924.

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that the mosaic roots were slower to develop seed stalks than the healthy, and, as the leaves appeared, all those from the mosaic roots were mottled. The mottling was most conspicuous on the older leaves, the younger growth in each case appearing healthy. The mosaic roots produced smaller tops than the healthy, and many of the blossoms dropped without developing pods. The average weight of dry seed produced by the plants grown from mosaic roots was 0.74 ounce, while the same number of plants from healthy roots averaged 1.85 ounces. The difference was chiefly due to the smaller number of pods on the mosaic plants. Counts and measurements showed that the individual pods on the mosaic plants were almost as large, and that the individual seeds were quite as large, as those on healthy plants, also, that the number of seeds per pod was almost the same on mosaic and healthy plants. The seed from healthy and diseased plants was kept separate and sown in three different localities in 1924. No mosaic appeared in two of these plantings all season and in the other only a few cases were observed toward the end of the season. The results indicate, therefore, that the disease is not carried in rutabaga seed, thus confirming the findings of Schultz with mustard.

HOST RANGE

The susceptibility to mosaic of turnips, rutabagas, mustard, and Chinese cabbage appeared to be well established, but the reaction of the more important crucifers, cabbage, cauliflower, and Brussels sprouts, was not known; consequently, inoculation experiments were undertaken with these crops. The cabbage aphid (*Brevicoryne brassicae* (L.)) was found to be exceptionally effective as an insect carrier and was used in most of the inoculation work. The green peach aphid (*Myzus persicae* (Sulz.)) was tried out twice with unsatisfactory results. Inoculation by hand consisted in rubbing crushed tissues from diseased plants into the leaves of healthy plants in such a way as to injure the midrib. This method was highly successful with the more susceptible host plants but not with those possessing a degree of resistance. The incubation period varied from three to more than five weeks and was shortest with the most susceptible host plants. Diseased rutabagas were the usual source of inoculum, and these proved very satisfactory. Rutabagas are easily infected and if kept in a cool place the diseased plants continue to make fair leaf growth. The cabbage aphid also multiplies freely on this host.

INOCULATION RESULTS IN THE GREENHOUSE

CHINESE CABBAGE (BRASSICA CHINENSIS L.).[6]—The plants were readily infected and showed inconspicuous mottling. At temperatures between 70° and 80° F. diseased plants were much stunted and developed streak symptoms. This plant was classed as highly susceptible.

MUSTARD, CULTIVATED WHITE (BRASSICA ALBA RABENH.) AND WILD BLACK (NIGRA KOCH).—The seed of the cultivated variety was purchased and that of the wild variety was collected from the fields. The plants of both were readily infected and reacted much like the

[6] The nomenclature followed is that used in Bailey's Manual of Cultivated Plants. BAILEY, L. H. MANUAL OF CULTIVATED PLANTS. 851 p., illus. New York. 1924.

Chinese cabbage. The leaves showed faint mottling and, at higher temperatures, plants were yellow and much stunted, usually developing streak. The mustards, while slightly less susceptible than the Chinese cabbage, were still highly susceptible.

FLAT TURNIP (BRASSICA RAPA L.).—The plants were readily infected and developed pronounced leaf mottling. At high temperatures diseased plants showed yellowing and stunting, but never developed streak. This plant was easily infected and susceptible, but was not as severely injured by the disease as mustard and Chinese cabbage.

RUTABAGA OR SWEDE (BRASSICA NAPOBRASSICA MILL.).—The behavior of this plant was similar to that of the flat turnip.

RAPE (BRASSICA NAPUS L.).—Several plants were inoculated in early November, 1925, with apparently negative results. They were held over winter, and one of them, about February 1, when flower shoots were forming, suddenly developed mottling that affected all of its leaves, and from then on it continued to show marked mosaic symptoms. The plants were growing in 3-gallon crocks and were very large at this time. The next fall six rape plants were inoculated and five showed distinct mosaic symptoms in four weeks; so the reason for the 3-months' incubation period of the previous year is not clear. In addition to mottling, the infected plants showed moderate stunting. The rape appeared to be somewhat less susceptible than the white turnip or rutabaga to which it is closely related.

BRUSSELS SPROUTS (BRASSICA OLERACEA L. VAR. GEMMIFERA ZENKER).—The early attempts to infect these plants were unsuccessful. As soon as the cabbage aphid was used as the inoculating agent, however, positive results were obtained, though the symptoms developed slowly. Occasional Brussels sprouts plants showed the usual type of mosaic mottling, but the great majority developed round yellow blotches that were scattered rather evenly over the leaves and were not raised. Later the tissues around the edges of the yellow areas died in a way that suggested the ring-spot disease of tobacco. Infected plants held at higher temperatures were stunted by the disease. At lower temperatures it was common for mosaic plants, after showing mosaic symptoms in half a dozen leaves, to recover and produce new leaves of normal appearance. None of the plants previously discussed have ever been observed to recover once they were infected, though the injury they suffered from the disease depended in part on environmental conditions.

The Brussels sprouts were distinctly more resistant to mosaic than the turnip or rape or any of the plants higher in the list.

CAULIFLOWER (BRASSICA OLERACEA L. VAR. BOTRYTIS L.).—As in the case of Brussels sprouts, early attempts to inoculate cauliflower failed but later efforts were successful. The symptoms that developed were similar to those on Brussels sprouts, with yellow spots scattered over the otherwise normal-appearing green leaf. However, in the case of the Brussels sprouts, the tissues around the edges of the spots died, and this did not occur in the cauliflower. Infected plants when placed under cool temperature conditions usually produced new leaves that showed no mosaic and, like the Brussels sprouts, outgrew the disease. During the course of the work a number of pure lines of cauliflower were inoculated by rubbing. In all lines but one, an occasional plant only developed mosaic symptoms and then of the usual mild character. Five plants of this one line were infected

and all showed the marked mottling and distortion of leaves such as occurs in turnips, indicating the possibility of mosaic-susceptible strains of cauliflower. The commercial cauliflower, however, was more resistant than the Brussels sprouts.

CABBAGE (BRASSICA OLERACEA L. VAR. CAPITATA L.).—Many plants were inoculated, but none developed positive symptoms of mosaic. A number of plants that developed doubtful symptoms were tested by using leaf tissues from them for back inoculating healthy rutabaga plants. The results of these tests were negative; hence, on the basis of the inoculation work conducted in the greenhouse, the cabbage was rated as immune to the cruciferous mosaic.

FIELD EXPERIMENTS AND OBSERVATIONS

On May 27, 1926, two beds were prepared and cauliflower, cabbage, Brussels sprouts, and rutabaga seeds were sown in separate rows. Two weeks later one bed was inclosed with cheesecloth and the plants were inoculated by allowing cabbage aphids taken from mosaic rutabagas to feed upon them. The aphids multiplied, and after 10 days all were killed with nicotine. On July 1, lots of 20 plants were set out from each seed-bed row from the inoculated bed and 4 from the check.

By August 20 the inoculated rutabagas were hardly a fifth the size of the uninoculated, and mosaic symptoms were well developed. The inoculated Brussels sprouts also had developed mosaic with the characteristic yellow leaf blotches. The inoculated cauliflower plants were not all growing as rapidly as the uninoculated, but none showed mosaic leaf symptoms. The inoculated and uninocualted cabbage plants were growing equally well with no visible differences. Typical mosaic symptoms on rutabaga, Brussels sprouts, and cauliflower leaves are shown in Figure 1.

By September 10, all 20 uninoculated rutabaga plants had contracted mosaic, though they were not yet stunted by the disease. All 20 of the inoculated Brussels sprouts plants showed the yellow mosaic spots, and around the borders of these were the circular rings of dead tissue peculiar to the disease on this plant. Two of the uninoculated Brussels sprouts now showed mosiac symptoms on the leaves, but whereas the 20 inoculated in the bed were much dwarfed these two later infected plants were still of normal size. Of the 20 inoculated cauliflower, 11 showed faint mosaic mottling at this time and these were all distinctly dwarfed. The remaining 9 inoculated were growing normally as were all 20 uninoculated. Three of the 20 inoculated cabbages showed faint markings that might indicate mosaic.

On October 1 the field experiment was concluded, and healthy greenhouse-grown rutabaga plants were inoculated by rubbing the leaves with crushed tissues from mosaic field-grown rutabaga, Brussels sprouts, and cauliflower plants. Three to five of the diseased field plants of each kind were selected and also the three cabbage plants that had shown possible mosaic symptoms. Two greenhouse rutabaga plants were inoculated with tissue from each field plant. Check pairs of rutabaga plants were inoculated with crushed tissues of healthy rutabaga, Brussels sprouts, cauliflower, and cabbage, these plants all being selected some distance away from any mosaic.

All six greenhouse rutabagas inoculated with tissues from mosaic field rutabagas developed mosaic. Of 10 greenhouse rutabagas,

FIGURE 1.—A, Mosaic symptoms on a rutabaga leaf. Affected leaves show yellowing and are ruffled and sometimes blistered; the mosaic pattern of light and dark patches is distinct and occasionally small necrotic areas develop. B, Mosaic symptoms on a Brussels sprouts leaf. Scattered yellow blotches appear on these leaves and somewhat later the tissues die at the edges of the spots forming "horseshoes." C, Mosaic symptoms on a cauliflower leaf. The mottling greatly resembles that appearing on Brussels sprouts leaves at an early stage of the disease; the numerous yellow blotches are very slightly raised and there is no leaf distortion nor necrosis

representing 5 mosaic field cauliflower plants, 8 developed mosaic and 2 remained healthy. The same numbers with Brussels sprouts gave 6 mosaic and 4 healthy. The 6 rutabaga plants inoculated from the 3 cabbages gave 2 mosaic (both inoculated from the same cabbage plant) and 4 healthy.

Mention has been made of the recovery of Brussels sprouts and cauliflower plants under greenhouse conditions. In the previous field experiment the Brussels sprouts plants that showed mosaic symptoms during the summer and were severely stunted began to improve about September 1, and by October 1 fully two-thirds of them had developed new and vigorously growing tops that appeared healthy. The same thing has been observed many other times in the field, and experienced growers, when shown diseased plants, have predicted that they would start to grow as soon as cool weather came. Mosaic cauliflower plants showed the same ability to recover under field conditions, but mosaic rutabagas, while making a better growth during cool weather, have always shown unmistakable symptoms of the disease. Another interesting field phenomenon that occurs rarely with either Brussels sprouts or cauliflower is the localization of the mosaic symptoms in the leaves on only one side of the plant.

OCCURRENCE OF DISEASE IN THE FIELD

The flat white turnip, rutabaga, Brussels sprouts, cauliflower, and cabbage are the common cultivated crucifers grown on Long Island and the black mustard is a prevalent weed. Mosaic has not been observed on white turnips. These are usually grown as a catch crop late in the summer in the market-gardening districts where the disease appears not to be established. Mosaic is common in the rutabaga fields of the Port Jefferson section, but has never caused serious loss. This is correlated with the fact that most of the infections occur late in the season and that the total infection in rutabaga fields has rarely exceeded 10 per cent. In Brussels sprouts fields in the East Marion section the mosaic disease has been commonly observed, the infection ranging from 5 per cent to a trace. In this locality black mustard is a common weed in and about Brussels sprouts fields and mosaic-diseased plants are frequently seen. Since the Brussels sprouts is a biennial and the mustard an annual, it appears likely that this is a case of a weed host contracting mosaic from a cultivated host. Many Brussels sprouts plants survive the winter in the fields. Mosaic is rare in cauliflower fields and 0.75 per cent is the maximum amount ever observed in a field.

No evidence of mosaic has ever been observed in cabbage fields, but the following instance points to cabbage as a possible carrier of the disease. A farmer who grew seed cabbage found his plants heavily infested with cabbage aphids at the time of cutting. As soon as the seed was harvested, the land was plowed and set with Brussels sprouts. A month later there was a general cabbage-aphid infestation, and over 80 per cent of the plants by actual count showed mosaic symptoms. This is about 15 times the maximum mosaic ever observed by the writer in any ordinary field of Brussels sprouts. The previous crop of aphid-infested cabbage appears to provide the only explanation of this outbreak of the disease, and the circumstantial evidence favors the idea that cabbage, while highly resistant to mosaic, may serve as a carrier.

DISCUSSION OF RESULTS

Since Brussels sprouts and cabbage are both hardy enough to live over winter unprotected on Long Island and in other localities where winters are not too severe, they offer a possible explanation of the method whereby the cruciferous mosaic carries over. At present no perennial weed hosts are known, and the tests with seed from mosaic rutabaga plants showed that the disease was not seed borne.

The cruciferous mosaic was found to be very responsive to environmental conditions and to injure the host plants most at high temperatures. (Fig. 2.) The susceptible Chinese cabbage and mustard developed the virulent streak form of mosaic when grown at a temperature of

FIGURE 2.—Relation between temperature and growth of healthy and mosaic-infected Chinese cabbage plants: A, Healthy (*a*) and infected (*b*) plants grown at 55° to 65° F.; B, healthy (*a*) and infected (*b*) plants grown at 70° to 80° F.

70° to 80° F. On the other hand, the less susceptible Brussels sprouts and cauliflower recovered completely when held at temperatures of 55° to 65°. The rapidity of field spread and the speed with which mosaic symptoms appeared varied directly with the susceptibility of the host plants, being greatest in both cases with the more susceptible. The cabbage aphid proved to be a very effective agent of dissemination in that it infected plants with great certainty. In the field, however, this insect is a very slow traveler. To what extent the green peach aphid is concerned in the spread of cruciferous mosaic is not certain. Schultz [7] reported that the insect was a good agent of dissemination, but in these tests it was very much less effective than the cabbage aphid.

[7] SCHULTZ, E. S. Op. cit.

Under Long Island conditions, with the crucifers grown as fall crops when the weather is cool, and with the more resistant of the crucifers only being cultivated, there is no reason to believe that this mosaic disease will ever be a serious problem. In the same locality it would appear likely that susceptible hosts such as Chinese cabbage and mustard if grown during midsummer might be seriously injured and it has been proved by actual test that such is the case.

SUMMARY

The mosaic disease of crucifers is a common but not serious disease of rutabagas on Long Island, N. Y. The disease has also been observed on Brussels sprouts, cauliflower, and black mustard.

Roots from diseased rutabagas held over winter threw up seed stalks which showed mosaic symptoms but matured seed. This seed was of normal appearance and the crop grown from it was healthy.

White and black mustards, Chinese cabbage, turnips, rutabagas, and rape were found to be susceptible hosts. Brussels sprouts and cauliflower, although susceptible, were not easily infected. Cabbage was either highly resistant or immune to the disease with some evidence that it may serve as a carrier.

Symptoms varied widely with the different host plants and also with environmental conditions.

At 70° to 80° F. the mustards and Chinese cabbage developed streak and the other plants were much stunted. At 55° to 65° even the more susceptible hosts made a fair development, while mosaic Brussels sprouts and cauliflower often ceased to show mosaic symptoms and grew normally. Under field conditions where these crops are sown in summer and develop during the fall it is usual for diseased Brussels sprouts and cauliflower to begin to recover about September 1.

It is conceded that cruciferous mosaic will continue to be a minor disease on Long Island because of the natural resistance of the most important economic crucifers, cabbage, cauliflower, and Brussels sprouts and also because these crops are grown during the cool weather of fall, whereas the disease develops best at high temperatures.

THE INSENSIBLE LOSS IN BODY WEIGHT OF CATTLE [1]

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INTRODUCTION

It is a fact of common knowledge that the total weight of the gaseous substances coming from the animal body always exceeds the weight of the gaseous intake; and the resulting insensible loss (often designated insensible perspiration) is one of the several factors responsible for the daily changes in the live weight of an animal, others being the quantities of food and water consumed and the weights of the visible excreta eliminated.

This subject has been extensively investigated with reference to human beings, and a historical account of such studies is given by Benedict and Root.[3] Although many factors affecting the insensible loss are recognized, Benedict and his coworkers [4] have found, in recent comprehensive experiments with human beings, that there exists a very close relationship between the insensible loss and the metabolic level. These researches have led the authors to the conclusion that the metabolism of human beings may be predicted with reasonable accuracy from the insensible loss, carefully determined under standard conditions.

Does a similar relationship exist in the case of cattle? Benedict and Ritzman [5] have recently reported data obtained with steers bearing on this question. These authors have found the evidence "sufficient to conclude that the most potent factor in determining the magnitude of the insensible perspiration is the general nutritive plane or the metabolic level. In other words, the insensible loss probably is loosely correlated with the total 24-hour metabolism of the animal at the time the insensible loss is measured." The authors call attention to the fact that the proof of the latter conclusion is lacking, since no simultaneous measurements of the 24-hour metabolism and of the insensible loss were made. They express, however, the belief that "the prediction of the total daily metabolism of steers may

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[2] The author is gratefully indebted to Dr. E. B. Forbes, director of this institute, for the encouragement received from him while preparing this paper and for the helpfulness of his criticisms. Acknowledgment is also made of the valuable assistance of H. H. Williams in checking some of the calculations.

[3] BENEDICT, F. G., and ROOT, H. F. INSENSIBLE PERSPIRATION: ITS RELATION TO HUMAN PHYSIOLOGY AND PATHOLOGY. Arch. Int. Med. 38: 1–35, illus.

[4] BENEDICT, F. G. BASAL METABOLISM: THE MODERN MEASURE OF VITAL ACTIVITY. Sci. Mo. 27: 5–27, illus. 1928.

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—— and ROOT, H. F. Op. cit.

[5] BENEDICT, F. G., and RITZMAN, E. G. THE METABOLISM OF THE FASTING STEER. p. 66–75. 1927. Washington, D. C. (Carnegie Inst. Wash. Pub. 377.)

actually be made with close approximation if the insensible loss, under controlled conditions of temperature, is accurately known."

An examination of the experimental records of this institute revealed the fact that in a large number of experiments with steers and cows data were available for the determination of the insensible loss, under controlled conditions, in the calorimeter, for periods during which the heat production was measured, and that data were also available for determining this loss in digestion trials under barn conditions.

The present paper, therefore, is devoted to computations of this insensible loss, and to a discussion of certain conditions of environment, the coat of hair of the animal, and the plane of nutrition, as they affect these values. The quantitative relation between the level of feeding and the insensible loss and the value of the latter as a basis for predicting the heat production are considered in a subsequent paper.[6]

CONDITIONS PREVAILING IN THE CALORIMETRIC EXPERIMENTS

In the metabolism experiments the animals were weighed on bullock scales (sensitive to 0.2 kgm.) immediately before entering the calorimeter and at the end of the calorimetric period. This period was 53 hours in length in the early experiments and from 85 to 87 hours in most of the more recent experiments. One calorimeter period considered, however (experiment 238, steer 36, period 12), was 111 hours in length. Records were also kept of the weights of feed and water consumed and of feces and urine eliminated during the period spent by the animal in the calorimeter. Data permitting the determination of the insensible loss were found available for 77 calorimetric periods, of which 58 were with steers, 11 with dry cows, and 8 with lactating cows.

The number of individual animals used as subjects in the 77 calorimetric experiments was 16, of which 4 were cows, and 12 were steers. The average live weights of the animals varied from 311 to 642 kgm.

The feed consumption in these experiments (except four fasting periods) varied between 392 and 2,268 gm. of dry matter per 100 kgm. of live weight per day. In 18 periods the rations consisted of roughage only, while in 55 periods they consisted of roughage and grain.

The temperature and the rate of ventilation were maintained uniform in the calorimeter during each of the experimental periods.

COMPUTATION OF THE INSENSIBLE LOSS OF BODY WEIGHT FROM THE LIVE WEIGHTS AND WEIGHTS OF FOOD, WATER, AND EXCRETA

The computation of the daily insensible loss from the daily live weights, the weights of the food and water consumed, and the weight of visible excreta is extremely simple, as will be seen from the following example: A steer weighed at 6 a. m. on October 1, 359.1 kgm. On October 2 at 6 a. m. he weighed 361.0 kgm. Between 6 a. m. October 1, and 6 a. m. October 2, he drank 16.1 kgm. of water, ate 6.51 kgm. of feed, and voided 6.69 kgm. of feces and 5.92 kgm. of

[6] KRISS, M. QUANTITATIVE RELATIONS OF THE DRY MATTER OF THE FOOD CONSUMED, THE HEAT PRODUCTION, THE GASEOUS OUTGO, AND THE INSENSIBLE LOSS IN BODY WEIGHT OF CATTLE. Jour. Agr. Research 40: pp. 283-295.

urine. Had it not been for the insensible loss the steer's weight at 6 a. m. on October 2 would have been $359.1+16.1+6.51-(6.69+5.92)=369.1$ kgm. The difference between 369.1 and 361.0—that is, 8.1 kgm.—is the insensible loss in body weight, and represents the sum of the weights of the water vapor, the carbon dioxide, and the methane produced, minus the weight of the oxygen consumed. Stated in different words, this insensible loss is made up of the carbon and the hydrogen of the fat, the proteins, and the carbohydrates oxidized in the body; the incompletely oxidized products (CH_4) excaping from the animal as a result of carbohydrate fermentation in the intestinal tract; and the water vaporized through the lungs and the skin. The latter factor usually makes up the greater portion of the total insensible loss.

The example given above is illustrative of the method of computing the daily insensible loss in the body weight of steers. The same method of computation is applicable to cows, except that in the case of lactating cows the weight of the daily milk produced should be properly taken into account as one of the egesta.

The average daily insensible loss for a period of several days can be obtained by either of the following procedures: (1) The insensible loss is computed for each day, the values obtained then being averaged; or (2) the total insensible loss for the period is computed, this loss being divided by the number of days represented.

For the computation of the average insensible loss by the first method the daily live weights, taken at the same time each day, must be known. The second method involves the use of the weights of the animal taken only at the beginning and end of the period. The same results are obtained by both methods, although the second method is much the shorter.

The daily insensible loss of the body weight of the animal in each of the calorimeter periods obtained by using the live weights and weights of foods, water, and excreta is given in Table 1. These values are referred to as the observed insensible loss to distinguish them from another set of values, also given in Table 1, which were obtained by a computation involving the use of the respiratory products, these latter values serving as checks on the former.

TABLE 1.—*A comparison of the directly observed insensible loss in body weight of cattle with that computed from the respiratory products*

Experiment and animal No.	Period No.	Length of calorimeter period	Insensible loss per day in calorimeter		Difference	
			Observed loss in weight	Computed from respiratory products	Computed minus observed	In per cent of observed
Experiment 240:		*Hours*	*Kgm.*	*Kgm.*	*Kgm.*	*Per cent*
Steer 60	1	85.5	3.3	3.7	+0.4	+12.1
57	2	86.5	4.5	4.6	+.1	+2.2
60	3	86.5	4.9	5.5	+.6	+12.2
57	4	87.0	6.3	6.8	+.5	+7.9
60	5	86.0	9.5	9.1	−.4	−4.2
57	6	86.0	10.4	10.9	+.5	+4.8
57	8	86.5	12.3	13.3	+1.0	+8.1
60	9	86.5	10.6	11.5	+.9	+8.5
57	10	86.5	4.0	4.3	+.3	+7.5
60	11	86.5	3.5	3.4	−.1	−2.9
57	12	86.0	6.4	6.1	−.3	−4.7
60	13	86.0	6.1	6.0	−.1	−1.6

TABLE 1.—*A comparison of the directly observed insensible loss in body weight of cattle with that computed from the respiratory products*

Experiment and animal No.	Period No.	Length of calorimeter period	Insensible loss per day in calorimeter		Difference	
			Observed loss in weight	Computed from respiratory products	Computed minus observed	In per cent of observed
		Hours	*Kgm.*	*Kgm.*	*Kgm.*	*Per cent*
Experiment 238:						
Steer 47	1	87.0	9.4	8.6	−.8	−8.5
36	2	87.0	9.4	8.9	−.5	−5.3
47	3	87.0	8.6	8.4	−.2	−2.3
36	4	87.0	7.8	8.3	+.5	+6.4
47	5	87.0	4.9	4.1	−.8	−16.3
36	6	87.0	5.0	4.0	−1.0	−20.0
47	7	87.0	6.0	5.6	−.4	−6.7
36	8	87.0	5.8	5.7	−.1	−1.7
36	10	87.0	6.7	7.2	+.5	+7.5
36 [a]	12	111.0	3.5	[b] 3.2	−.3	−8.6
Experiment 237:						
Steer 47	2	85.0	5.1	6.7	+1.6	+31.4
254	3	85.5	4.0	4.0	0	0
47	4	85.0	5.9	5.9	0	0
254	5	85.5	6.2	5.8	−.4	−6.5
47	6	85.5	6.5	6.1	−.4	−6.2
254	8	85.0	6.7	6.2	−.5	−7.5
36	10	86.0	5.2	5.6	+.4	+7.7
47	11	85.5	6.0	5.8	−.2	−3.3
36	12	86.0	4.7	4.7	0	0
47	13	86.0	4.7	4.9	+.2	+4.3
Experiment 235:						
Steer 259	2	86.0	4.5	[b] 4.0	−.5	−11.1
260	2	85.5	6.4	[b] 6.0	−.4	−6.2
259	3	86.0	6.9	[b] 6.5	−.4	−5.8
260	3	86.0	8.9	[b] 8.4	−.5	−5.6
Experiment 220:						
Steer K	1	53.0	6.1	6.4	+.3	+4.9
K	3	53.0	5.9	5.6	−.3	−5.1
K	4	53.0	9.4	8.9	−.5	−5.3
Experiment 217:						
Steer J	1	53.0	6.6	6.0	−.6	−9.1
J	2	53.0	17.8	16.7	−1.1	−6.2
J	4	53.0	9.6	9.5	−.1	−1.0
Experiment 236:						
Steer J	1	53.0	13.3	12.6	−.7	−5.3
J	2	53.0	5.8	5.5	−.3	−5.2
J	3	53.0	7.7	6.7	−.1	−13.0
J	4	53.0	4.4	3.9	−.5	−11.4
J	5	53.0	9.6	8.9	−.7	−7.3
J	6	53.0	7.5	6.9	−.6	−8.0
J	7	53.0	4.8	4.3	−.5	−10.4
Experiment 212:						
Steer H	3	53.0	5.8	5.0	−.8	−13.8
H	4	53.0	5.4	5.1	−.3	−5.6
Experiment 211:						
Steer D	1	53.0	6.2	6.2	0	0
D	2	53.0	4.8	4.8	0	0
D	5	53.0	2.5	3.2	+.7	+28.0
G	2	53.0	4.4	3.9	−.5	−11.4
G	3	53.0	10.7	10.2	−.5	−4.7
G	4	53.0	5.0	4.7	−.3	−6.0
G	5	53.0	3.0	3.1	+.1	+3.3
Experiment 221 D:						
Cow 886	2	53.0	9.2	8.8	−.4	−4.3
886	3	53.0	5.0	4.8	−.2	−4.0
885	3	53.0	6.2	6.3	+.1	+1.6
Experiment 221 E:						
Cow 886	2	53.0	7.2	6.1	−1.1	−15.3
874	1	53.0	8.5	8.0	−.5	−5.9
874	2	53.0	7.7	7.2	−.5	−6.5
885	2	53.0	6.7	5.9	−.8	−11.9
885 [a]	3	53.0	2.0	[b] 2.4	+.4	+20.0
Experiment 221 F:						
Cow 886	2	53.0	8.0	7.9	−.1	−1.2
874	1	53.0	8.4	[b] 7.8	−.6	−7.1
874	2	53.0	4.6	[b] 4.4	−.2	−4.3
874 [a]	3	53.0	2.2	[b] 2.5	+.3	+13.6
887	1	53.0	6.2	[b] 7.0	+.8	+12.9
887	2	53.0	4.0	[b] 4.5	+.5	+12.5
887 [a]	3	53.0	2.3	[b] 2.0	−.3	−13.0
Experiment 221 G:						
Cow 887	1	53.0	7.9	7.6	−.3	−3.8
887	2	53.0	3.7	3.8	+.1	+2.7
887	3	53.0	5.7	5.4	−.3	−5.3
887	4	53.0	8.5	8.1	−.4	−4.7

[a] Fasting. [b] Oxygen consumption was determined.

COMPUTATION OF THE INSENSIBLE LOSS OF BODY WEIGHT FROM THE RESPIRATORY PRODUCTS

In all the calorimetric experiments the water vapor, the carbon dioxide, and the methane production of the animals were quantitatively accounted for. Determinations of the oxygen consumption, however, were made in only 12 of the 77 periods. In these 12 periods it was possible to compute the insensible loss by subtracting the weight of the oxygen consumed from the sum of the weights of the water vapor, carbon dioxide, and methane produced. The values so computed are indicated in Table 1 by footnote reference *b*. This computation, obviously, could not be applied to the remaining 65 periods in which direct determinations of the oxygen consumption were lacking. It was possible, however, in these experiments, to make use of a modification of this method for computing the insensible loss by taking into consideration the gains or losses of body tissue and the extent as well as the source of oxygen used.

It is clear that the O_2 of the CO_2 does not correctly represent atmospheric oxygen consumed, because some of this O_2 might be derived from carbohydrates (as in the synthesis of fat), while, on the other hand, some of the atmospheric O_2 consumed might go to form H_2O (as in the oxidation of fat). However, the amounts of nonatmospheric oxygen excreted in CO_2 and the amount of atmospheric O_2 appearing as H_2O can be computed from the observed gains and losses of body tissue.

For the purpose of this computation it seemed permissible to neglect the katabolism of protein and to consider only the nonnitrogenous material oxidized. If this consisted only of carbohydrates—the respiratory quotient, therefore, being 1.0—the O_2 of the CO_2 could be considered as accurately representing the atmospheric oxygen consumed; but if some of the material oxidized consisted of fat, all the oxygen contained in the carbon dioxide as well as some of the oxygen of the water eliminated would have been derived from the air. On the other hand, if a formation of fat from carbohydrates took place, some of the oxygen of the carbon dioxide would have been derived from the carbohydrates consumed. On the basis of the average composition of animal fat, viz, C = 76.5 per cent, H = 12.0 per cent, O = 11.5 per cent, the following equivalents were computed:

Oxidation of 100 gm. of fat

	Gm.
O_2 required for oxidation of H_2	96. 0
O_2 contained in fat	11. 5
O_2 derived from the atmosphere	84. 5

Formation of 100 gm. of fat

	Gm.
$C_6H_{12}O_6$ required to furnish C	191. 25
O_2 in 191.25 gm. of $C_6H_{12}O_6$	102. 0
O_2 contained in 100 gm. of fat	11. 5
O_2 of carbohydrate excreted in CO_2	90. 5

In accord with the foregoing values, if to the carbon of the carbon dioxide produced is added the gain of fat multiplied by 0.905, or if from the carbon of the carbon dioxide is subtracted the loss of fat

multiplied by 0.845, the result represents the difference in weight between the CO_2 produced and the oxygen consumed. By adding to the latter result the weights of the water vapor and of the methane produced, the total insensible loss is obtained. This procedure was employed in the derivation of 65 of the computed values of insensible loss as recorded in Table 1.

COMPARISON OF THE DIRECTLY OBSERVED INSENSIBLE LOSS IN BODY WEIGHT WITH THAT COMPUTED FROM THE RESPIRATORY PRODUCTS

An examination of the data in Table 1 reveals the fact that the insensible loss as computed from the respiratory products agrees fairly well with the observed loss in body weight. The agreement between the computed and the observed values is just as close in the cases where the oxygen consumption was directly determined as in the cases where no such determinations of oxygen were made. This agreement indicates that there are, at the most, no gross errors in the values for insensible loss obtained by either of the methods used. This may also signify that it is possible to compute the O_2 consumption with a fair degree of accuracy when the CO_2 production and the gain or loss of fat are known. In the case of ruminants, however, it is quite conceivable that some of the oxygen of the carbon dioxide produced is derived from carbohydrates by process of fermentation. There is, however, no satisfactory basis available for the estimation of this quota.

It will be observed that the insensible loss in these experiments varies considerably in magnitude, the lowest observed value being 2.0 kgm. and the highest 17.8 kgm. per day. The computed values agree fairly well with the observed, being slightly larger in some cases and smaller in others. The lowest computed value is 2.0 kgm. and the highest is 16.7 kgm. per day. These large variations, as is shown later, appear to be due chiefly to differences in the plane of nutrition and in the size of the animals.

INFLUENCE OF ENVIRONMENTAL CONDITIONS ON THE INSENSIBLE LOSS OF BODY WEIGHT

In addition to the determinations of the insensible loss during the calorimeter periods it was found possible to determine such values, though with somewhat less accuracy, in 71 associated digestion periods, the animals being confined in stalls, in a barn, under less rigidly controlled conditions as to temperature and ventilation than in the calorimeter. A slight element of error in these values resulted from the fact that in some cases the excreta were collected at 6 a. m., while the animal was not weighed until about 8 a. m. Also, the animal was fed at 6 a. m., and no record was kept of any feed which might have been left in the manger at the time the animal was weighed. In computing the daily insensible loss it was necessary to assume that both the feed box and the boxes for collecting the excreta were empty at the time the animal was weighed. It would seem that such an assumption might cause considerable error in the daily determination of the insensible loss. However, in the determination of the

average daily insensible loss for an experimental period it is not necessary to consider all the daily weighings of the animal or the time of such weighings; in fact, it is necessary to consider only the initial and final live weights. The digestion periods varied in length from 5 to 14 days. Hence, any error that might have arisen from the situation discussed could be, at the most, but a small fraction of the total insensible loss.

Of the 71 digestion experiments mentioned, 8 were with dry cows, 8 with lactating cows, and 55 with steers. The number of individual animals used in all these experiments was 16, of which 4 were cows, and 12 were steers.

In 24 of these digestion periods a continuous record of the temperature of the barn was obtained. Since the animals received rations of the same quantities and character during the digestion periods outside the calorimeter as during the associated periods in the calorimeter, it was possible to compare the insensible loss as determined under these different environmental conditions. Such a comparison is made in Table 2.

The average temperature in the calorimeter was higher than the average temperature in the digestion stall in all but one of the periods for which continuous records of temperature in the stall were available. It will be observed that the differences in insensible loss were not always in the same direction as the differences in temperature. In the one case (experiment 240, steer 60, period 9) in which the temperature in the calorimeter was 2.2° C. lower than the temperature in the stall, the insensible loss in the calorimeter was considerably less than the insensible loss in the stall. In six cases the insensible loss in the calorimeter was less than the corresponding insensible loss in the stall, although the temperature in the calorimeter was the higher. In 16 cases the relatively higher temperature in the calorimeter was attended by a higher insensible loss.

All the differences in insensible loss recorded in Table 2 may be viewed as representing the aggregate effect of the differences between the environmental conditions in the calorimeter and in the digestion stall. The temperature of the air in the calorimeter was always well controlled, and, as shown in the table, was with one exception appreciably higher than the recorded average temperatures in the digestion stalls in the barn. In those periods for which stall temperatures are missing, the average temperatures in the stall may be assumed to have been in most cases lower than those in the calorimeter, in view of the high temperatures maintained in the latter. The calorimeter temperatures given in the table are averages of readings taken at half-hour intervals, while the temperatures of the stall are averages of readings taken at 3-hour intervals. The temperatures of the calorimeter were quite constant, while those of the stall varied considerably during the experimental period. No observations were made on the humidity either in the stall or in the calorimeter, but in all probability differences in this respect existed. Air was admitted to the calorimeter at a definite rate, and it was agitated by means of an electric fan. The rations, however, were the same in both the stall and the calorimeter.

TABLE 2.—*Influence of environmental conditions and of plane of nutrition on the insensible loss in body weight of cattle*

Experiment and animal No.	Period No.	Average live weight of animal	Dry matter of feed consumed per day	Results obtained in digestion stall			Results obtained in calorimeter			Difference in temperature (temperature in calorimeter minus temperature in stall)	Difference in insensible loss	
				Number of days averaged	Average temperature of air	Average insensible loss per day	Rate of ventilation per minute	Average temperature of air	Average insensible loss per day		Insensible loss in calorimeter minus insensible loss in stall	In per cent of insensible loss in calorimeter
		Kgm.	*Kgm.*		*° C.*	*Kgm.*	*Liters*	*° C.*	*Kgm.*	*° C.*	*Kgm.*	*Per cent*
Experiment 240:												
Steer 60	1	311	2.8	14	------	3.5	443	17.7	3.3	------	−0.2	−6.1
57	2	360	3.1	12	------	3.9	446	17.6	4.5	------	+.6	+13.3
60	3	333	4.2	10	------	4.2	446	17.6	4.9	------	+.7	+14.3
57	4	384	4.6	13	14.9	7.0	446	17.5	6.3	+2.6	−.7	−11.1
60	5	358	5.7	12	14.7	9.4	440	17.6	9.5	+2.9	+.1	+1.1
57	6	403	6.2	12	15.3	12.7	444	17.5	10.6	+2.2	−2.1	−19.8
57	8	444	8.1	14	14.8	14.5	598	15.9	12.3	+1.1	−2.2	−17.9
60	9	427	9.5	13	14.1	16.5	614	11.5	10.6	−2.2	−5.9	−55.7
57	10	398	1.7	13	13.7	4.3	444	17.5	4.0	+3.4	−.3	−7.5
60	11	381	1.7	12	12.6	4.4	445	17.3	3.5	+4.7	−.9	−25.7
57	12	456	5.2	12	13.2	4.2	441	17.3	6.4	+4.1	+2.2	+34.4
60	13	412	5.0	14	13.7	5.5	441	17.2	6.1	+3.5	+.6	+9.8
Experiment 221 D:												
Cow 886	2	424	6.1	8	8.6	6.4	675	17.6	9.2	+9.0	+2.8	+30.4
886	3	420	3.8	8	12.2	4.0	670	17.5	5.0	+5.3	+1.0	+20.0
886	3	426	3.9	8	12.2	4.6	677	17.6	6.2	+5.4	+1.6	+25.8
885												
Experiment 221 E:												
Cow 886	2	401	7.0	8	13.8	6.4	575	17.7	7.2	+3.9	+.8	+11.1
874	1	381	6.9	8	13.3	6.4	673	18.0	8.5	+4.7	+2.1	+24.7
874	2	391	6.6	8	14.5	7.7	607	18.2	7.7	+3.7	.0	.0
874	2	434	3.4	7	------	4.3	579	17.9	6.7	------	+2.4	+35.8
885												
Experiment 221 F:												
Cow 886	2	412	8.1	8	17.1	9.1	489	18.1	8.0	+1.0	−1.1	−13.8
874	1	429	6.0	8	11.7	6.3	497	17.4	8.4	+5.7	+2.1	+25.0
874	2	416	4.0	8	9.0	4.1	498	17.8	4.6	+8.8	+.5	+10.9
887	1	335	5.4	8	8.7	4.5	488	17.8	6.2	+9.1	+1.7	+27.4
887	2	320	3.6	7	11.0	3.3	498	17.7	4.0	+6.7	+.7	+17.5
Experiment 220:												
Steer K	1	514	6.0	6	------	7.0	652	17.8	6.1	------	−.9	−14.8
K	3	490	3.9	5	------	7.3	641	17.1	5.9	------	−1.4	−23.7
K	4	514	6.6	5	------	8.9	652	18.3	9.4	------	+.5	+5.3
Experiment 217:												
Steer J	1	490	4.5	6	------	6.5	647	18.3	6.6	------	+.1	+1.5
J	2	536	9.1	6	------	18.8	644	18.4	17.8	------	−1.0	−5.6
J	4	642	5.2	5	------	9.5	649	18.1	9.6	------	+.1	+1.0
Experiment 216:												
Steer J	1	389	8.8	6	------	12.3	650	17.8	13.3	------	+1.0	+7.5
J	2	367	3.8	5	------	4.3	654	17.7	5.8	------	+1.5	+25.9
J	3	387	5.3	6	------	7.5	654	17.7	7.7	------	+.2	+2.6
J	5	404	7.9	6	------	7.9	653	17.7	9.6	------	+1.7	+17.7
J	7	377	3.5	6	------	4.4	652	17.7	4.8	------	+.4	+8.3
Experiment 212:												
Steer H	3	354	5.3	6	------	6.2	652	17.9	5.8	------	−.4	−6.9
H	4	349	5.4	6	------	5.6	657	17.8	5.4	------	−.2	−3.7
Experiment 211:												
Steer D	1	460	6.2	6	------	7.4	679	17.9	6.2	------	−1.2	−19.4
D	2	432	3.5	5	------	4.9	650	17.9	4.8	------	−.1	−2.1
D	3	470	7.9	6	------	10.6	644	17.9	12.8	------	+2.2	+17.2
D	5	428	1.8	6	------	4.5	672	17.8	2.5	------	−2.0	−80.0
D	2	358	2.3	6	------	3.7	654	17.9	4.4	------	+.7	+15.9
G	3	398	7.0	6	------	12.7	637	17.9	10.7	------	−2.0	−18.7
G	4	387	3.1	6	------	6.0	663	17.8	5.0	------	−1.0	−20.0
G	5	364	1.8	6	------	3.5	666	17.7	3.0	------	−.5	−16.7
Experiment 221 G:												
Cow 887	1	383	8.0	9	12.4	7.0	492	18.0	7.9	+5.6	+.9	+11.4
887	2	346	3.8	8	10.9	2.3	491	17.6	3.7	+6.7	+1.4	+37.8
887	3	345	5.9	8	10.8	4.4	493	17.8	5.7	+7.0	+1.3	+22.8
887	4	358	8.0	8	6.8	5.5	492	17.9	8.5	+11.1	+3.0	+35.3
Experiment 238:												
Steer 47	1	486	7.4	14	------	9.9	629	8.9	9.4	------	−.5	−5.3
36	2	483	7.0	14	------	9.8	621	9.7	9.4	------	−.4	−4.3
47	3	495	5.6	14	------	7.8	613	14.4	8.6	------	+.8	+9.3
36	4	490	5.4	14	------	7.0	617	14.4	7.8	------	+.8	+10.3
47	5	475	1.9	8	------	4.0	453	16.7	4.9	------	+.9	+18.4
36	6	471	1.9	9	------	4.0	448	16.7	5.0	------	+1.0	+20.0
47	7	485	3.8	14	------	4.5	448	16.7	6.0	------	+1.5	+25.0
36	8	481	3.8	14	------	4.7	450	16.7	5.8	------	+1.1	+19.0
36	10	500	5.8	14	------	5.1	608	16.9	6.7	------	+1.6	+23.9

TABLE 2.—*Influence of environmental conditions and of plane of nutrition on the insensible loss in body weight of cattle*—Continued

Experiment and animal No.	Period No.	Average live weight of animal	Dry matter of feed consumed per day	Results obtained in digestion stall			Results obtained in calorimeter			Difference in temperature (temperature in calorimeter minus temperature in stall)	Difference in insensible loss	
				Number of days averaged	Average temperature of air	Average insensible loss per day	Rate of ventilation per minute	Average temperature of air	Average insensible loss per day		Insensible loss in calorimeter minus insensible loss in stall	In per cent of insensible loss in calorimeter
Experiment 237:		*Kgm.*	*Kgm.*		*° C.*	*Kgm.*	*Liters*	*° C.*	*Kgm.*	*° C.*	*Kgm.*	*Per cent*
Steer 47	2	360	4.0	11	.....	6.7	447	17.5	5.1	.....	−1.6	−31.3
254	3	345	2.0	9	.....	3.9	445	17.6	4.0	.....	+.1	+2.5
47	4	362	3.4	14	.....	6.4	444	17.5	5.9	.....	−.5	−8.5
254	5	347	3.3	12	.....	4.4	451	17.7	6.2	.....	+1.8	+29.0
47	6	357	4.0	8	.....	5.3	442	17.5	6.5	.....	+1.2	+18.5
36	10	330	4.0	10	.....	5.9	445	17.6	5.2	.....	−.7	−13.5
47	11	355	4.4	7	.....	6.8	435	17.6	6.0	.....	−.8	−13.3
36	12	319	3.5	14	.....	5.1	443	17.7	4.7	.....	−.4	−8.5
47	13	347	3.8	14	.....	3.3	443	17.6	4.7	.....	+1.4	+29.8
Experiment 235:												
Steer 259	2	351	3.5	14	.....	3.9	446	17.7	4.5	.....	+.6	+13.3
260	2	369	3.5	14	.....	5.5	441	17.7	6.4	.....	+.9	+14.1
259	3	379	5.7	14	.....	6.5	445	17.7	6.9	.....	+.4	+5.8
260	3	398	5.7	14	.....	8.6	443	17.7	8.9	.....	+.3	+3.4

Of the 71 comparisons presented in Table 2, 44 showed a greater insensible loss in the calorimeter than in the digestion stall and 26 showed a greater loss in the stall than in the calorimeter.

The above observations indicate that the temperature of the environment tends to exert a definite influence on the insensible loss, but that this influence is not very pronounced, or is modified by other conditions, such as the movement of the air and the relative humidity.

INFLUENCE OF THE COAT OF HAIR ON THE INSENSIBLE LOSS OF BODY WEIGHT

In connection with a consideration of the possible influences of the environmental conditions on the insensible loss it seems to be relevant to consider the coat of hair of the animal. No detailed study of the influence of this factor has been made, but some information can be gained by referring to experiment 235 in Table 2.

In this experiment steers 259 and 260, which were of nearly the same size, received identical rations in the corresponding periods. In period 2 each of the two steers received a maintenance ration, and in period 3 each received a supermaintenance ration. Steer 259 was shorn, while steer 260 possessed his full winter coat. In both periods 2 and 3 the insensible loss of the steer having his full coat of hair was considerably greater than that of the steer which had been shorn. In period 2 the insensible loss of steer 260, in the barn, exceeded the corresponding loss of steer 259 by 1.6 kgm.; and in period 3 this excess was 2.1 kgm. In the calorimeter, under identical conditions as to temperature and ventilation, the insensible loss of steer 260 exceeded the loss of No. 259 by 1.9 kgm. in period 2 and by 2.0 kgm. in period 3. In this experiment, at least, the influence of the coat of hair appeared

to be considerably greater than the aggregate effect of the differences in environmental conditions in the barn as compared with those in the calorimeter.

INFLUENCE OF PLANE OF NUTRITION ON THE INSENSIBLE LOSS OF BODY WEIGHT

The data in Table 2 afford excellent means for studying the effect of the plane of nutrition on the insensible loss. Since the environmental conditions were nearly uniform in the calorimeter, fairly correct information regarding the influence of the nutritive plane on the insensible loss may be had by comparing the amounts of feed received by the individual animals with the values for insensible loss obtained in the calorimeter. In making such comparisons it is necessary also to consider the live weights of the animals.

It will be observed that in general, the insensible loss is closely related to the amounts of feed consumed. In nearly every experiment the lowest amounts of feed consumed by the individual animals were accompanied by the lowest values for insensible loss, and the highest amounts of feed were accompanied by the highest values for insensible loss.

The size of the animals also affects the magnitude of these values. This can best be seen by comparing the values for insensible loss of individuals receiving identical amounts of feed but varying considerably in size. Thus, in experiment 216, period 3, the insensible loss of body weight of steer J receiving 5.3 kgm. of feed and weighing 387 kgm. was 7.7 kgm. in the calorimeter as compared with 9.6 kgm. for the insensible loss of the same steer in experiment 217, period 4, on practically the same amount of feed (5.2 kgm.) but weighing 642 kgm. Similarly, it is found that the insensible loss in the calorimeter of steers 36 and 47 was greater in experiment 238, periods 7 and 8, than the values for the same steers on practically the same amounts of feed in experiment 237, periods 12 and 13, the steers being considerably heavier in experiment 238 than in experiment 237.

It is apparent, therefore, that the large variations noted in the values for insensible loss in the different periods both in the calorimeter and in the digestion stall are mainly due to differences in the plane of nutrition and in the size of the animals, the feed consumption showing the most pronounced effects.

The quantitative relation between the plane of nutrition and the insensible loss, and the value of the insensible loss as a basis for computing the heat production, are considered in a subsequent paper.[7]

SUMMARY

In this paper are presented determinations of the average daily insensible loss in body weight (excess of gaseous outgo over gaseous income) of cattle and a discussion of some factors which affect this value.

Data from 77 respiration calorimetric experiments, varying in length from 53 to 111 hours, and from 71 digestion trials of 5 to 14 days' duration, were used as the basis for the computation of the insensible

[7] Kriss, M. Op. cit

loss of steers and cows, at planes of nutrition varying from fast to about three times the maintenance requirement.

The insensible loss in body weight was computed in all experiments by a procedure based on the live weights of the animals, the weights of the feed and water consumed, and the weights of the excreta voided; and, in addition, in some experiments, the results thus obtained were checked by a computation based on the respiratory products as measured in the calorimeter. There was good agreement between the values for the insensible loss as obtained by the two methods.

The insensible losses in the calorimetric experiments varied considerably in magnitude. The lowest observed value (based on live weights) was 2.0 kgm. per day, and the highest 17.8 kgm. per day. The lowest computed value (based on the respiratory products) was 2.0 kgm. per day, and the highest 16.7 kgm. per day. These large variations are related to the differences in planes of nutrition and size of the animals.

A comparison of the data for the average daily insensible loss obtained in the digestion stall with those obtained in the calorimeter indicates that the temperature of the environment exerts a definite influence on the insensible loss, but that this influence is either not very pronounced or is modified by other conditions of the environment, such as the movement of the air and the relative humidity.

The data indicate that the coat of hair influences the insensible loss in a marked degree.

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QUANTITATIVE RELATIONS OF THE DRY MATTER OF THE FOOD CONSUMED, THE HEAT PRODUCTION, THE GASEOUS OUTGO, AND THE INSENSIBLE LOSS IN BODY WEIGHT OF CATTLE[1]

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INTRODUCTION

The methods necessary to the investigation of the principles of respiration and energy metabolism of man and animals—even the simpler methods of indirect calorimetry—are sufficiently complicated to preclude their general use in the solution of problems of a practical nature. This is as true in relation to animals as to human beings. As the facts relating to the energy metabolism of animals become more clearly established, however, the underlying principles come to light, and make possible the application of the fundamental truths to practical problems of animal metabolism.

One such problem—the ventilation requirements in barns—requires, for its study, a knowledge of the respiratory metabolism and the heat production of farm animals. Stables as a rule are not artificially heated, the heat supplied by the animals alone serving to temper the cold of winter weather. Another important function of the animal heat is to serve as a motive power in ventilation. The water vapor, which comes from the lungs and skin of the animals, adds to the humidity, while the carbon dioxide and the methane given off serve as continuous contaminations of the stable air. All these factors affect the comfort and, presumably, the productiveness of the animals.

Obviously, it is impracticable to employ a respiration apparatus directly in the study of this subject. It is important, therefore, to establish a method for the consideration of the matter by a process of computation. In 1921 Armsby and Kriss (*1*)[3] proposed a method based on respiration calorimetric data, for computing the heat production, water vapor, and carbon dioxide production of farm animals, with approximate accuracy, from the live weights of the animals and the rations received. According to this method the heat production of cattle is obtained by adding to the computed maintenance requirement of net energy the total heat increment (energy cost of food utilization) of the ration, this quota being estimated on

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[3] Reference is made by number (italic) to "Literature cited," p. 295.

the basis of average heat-increment values; while the CO_2 production is computed from the heat-CO_2 ratio of Armsby, Fries, and Braman (*2*).

It is unfortunate that the number of experimentally determined heat-increment values of feeding stuffs is very small, even for cattle. This fact considerably limits the usefulness of the method referred to; and a modification seems desirable, as has been recently suggested by Kelley (*7*).

In an effort to provide an adequate basis for the computation of the amounts of the heat production and the gaseous products of cattle, for practical uses, especially as applying to the solution of problems of ventilation of barns, the writer has made a biometric study of the measurements indicated below, all of which were derived from published respiration calorimetric experiments of this institute.

This study has been directed to the establishment of the relationship between (1) the dry matter of the feed consumed and the insensible loss; (2) the insensible loss and the heat production; (3) the dry matter of the feed consumed and the heat production; (4) the carbon dioxide and the heat production; (5) the water vapor and the heat production; (6) the dry matter of the feed consumed and the carbon dioxide production; (7) the dry matter of the feed consumed and the water vaporized; and (8) the dry matter of the feed consumed and the methane production.

DATA SUBJECTED TO STATISTICAL ANALYSIS

The data analyzed comprise two series.

FIRST SERIES

For the purpose of determining the correlation between the dry matter of the food consumed and the insensible loss, and the correlation between the insensible loss and the heat production, the results of 74 experimental periods were used, including all except the fasting periods indicated in Table 1 of the foregoing paper (*8*). Of these 74 experiments, 58 were with steers, 8 with dry cows, and 8 with lactating cows. The number of individual animals used as subjects in these experiments was 16, of which 4 were cows and 12 were steers. The average live weights of the animals varied from 311 to 642 kgm. The feed consumption in these experiments varied from 392 to 2,268 gm. of dry matter per 100 kgm. of live weight per day. In this series the following measurements were considered: (1) Insensible loss per 100 kgm. of live weight per day; (2) heat production per 100 kgm. of live weight per day; and (3) dry matter of feed eaten per 100 kgm. of live weight per day.

The data for insensible loss used in this analysis are those obtained under the well-controlled conditions in the respiration calorimeter. The values for heat production of this, as well as of the following series, are as directly observed—uncorrected for difference in time spent standing and lying.

SECOND SERIES

This series comprises the results of 131 experimental periods, which include 66 of the first series (excluding the experiments with lactating cows). Of the total number of experiments 123 were with steers and 8 with dry cows. The number of individual animals used as subjects

in these experiments was 24, of which 20 were steers and 4 were cows. The average live weight of the animals varied from 162 to 655 kgm. The feed consumption varied from 392 to 2,268 gm. of dry matter per 100 kgm. of live weight per day. In this series the following measurements were considered: (1) Heat production per 100 kgm. of live weight per day; (2) carbon dioxide production per 100 kgm. of live weight per day; (3) water vaporized per 100 kgm. of live weight per day; (4) dry matter of feed eaten per 100 kgm. of live weight per day; (5) dry matter of feed eaten per head per day; and (6) methane production per head per day.

METHODS AND RESULTS OF STATISTICAL ANALYSIS

The statistical methods used are those of the standard text books on biometry, but particularly the methods and formulas discussed and used by Harris and Benedict in their Biometric Study of Basal Metabolism in Man (*6*).

The main results sought in this analysis were coefficients of correlation between the various measurements considered, and regression equations. The computations of the statistical constants, such as the means, the standard deviations and the coefficients of variation, are incidental to the main purpose of this analysis.

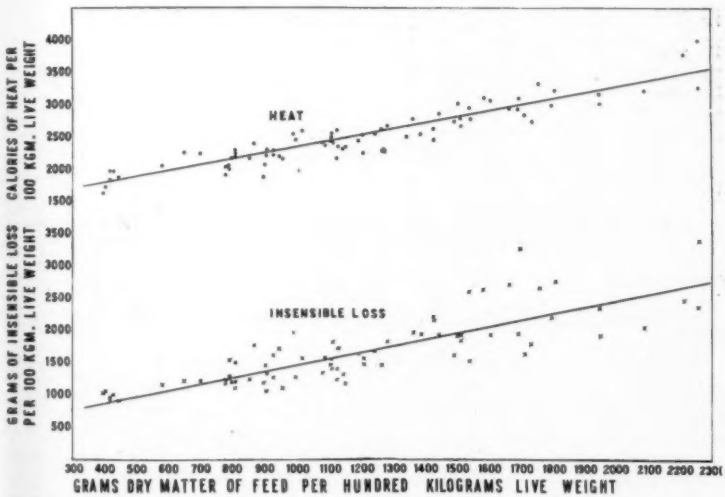
CALORIES OF HEAT PER 100 KGM. LIVE WEIGHT
GRAMS OF INSENSIBLE LOSS PER 100 KGM. LIVE WEIGHT
HEAT
INSENSIBLE LOSS
4000
3500
3000
2500
2000
1500
1000
500
300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500 1600 1700 1800 1900 2000 2100 2200 2300
GRAMS DRY MATTER OF FEED PER HUNDRED KILOGRAMS LIVE WEIGHT

FIGURE 1.—Relationships (1) between dry matter of feed consumed and heat production and (2) between dry matter of feed consumed and insensible loss in body weight of cattle

All the data analyzed are represented graphically in Figures 1 to 5. The data of the first series are shown in Figures 1 and 2. In Figure 1 the insensible loss and the heat production are plotted against the dry matter of feed consumed, or, more specifically, the grams of dry matter of feed per 100 kgm. of live weight were used as abscissas, and the grams of insensible loss and Calories of heat per 100 kgm. of live

weight were used as ordinates. This, therefore, exhibits diagramatically the relation between the dry matter of feed consumed and the insensible loss, and the relation between the dry matter of feed consumed and the heat production of the animal. In Figure 2 the heat production is plotted against the insensible loss, and the relationship between these two measurements is shown.

The statistical analysis of the data represented by Figures 1 and 2 reveals that there is a fairly close correlation between the dry matter of the feed consumed and the insensible loss and between insensible loss and heat production, but that there exists a considerably closer correlation between the dry matter of the feed consumed and the heat production than between the insensible loss and the heat production. The following correlation coefficients were obtained: Between dry matter of feed and insensible loss, 0.818 ± 0.026; between insensible loss and heat production, 0.847 ± 0.022; and between dry matter of feed and heat production, 0.936 ± 0.010.

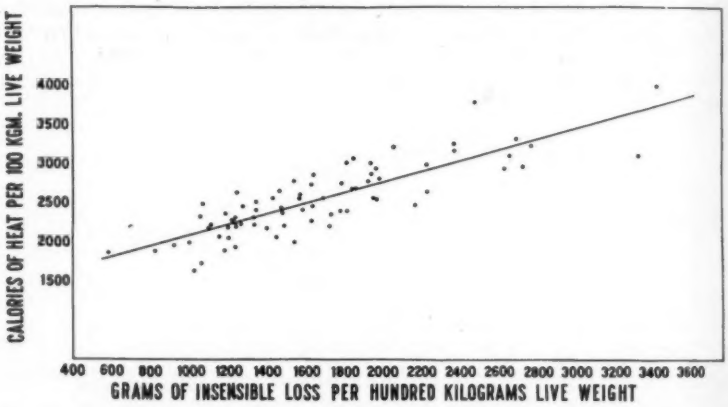

FIGURE 2.—Relationship between the insensible loss in body weight and the heat production of cattle

Figures 3, 4, and 5 represent data of the second series referred to above.

In Figure 3 the carbon dioxide production, water vapor, and heat production are plotted against the dry matter of the feed consumed. These data gave upon analysis the following coefficients of correlation: Between dry matter of feed and heat production, 0.883 ± 0.013; between dry matter of feed and CO_2 production, 0.938 ± 0.007; and between dry matter of feed and water vapor, 0.762 ± 0.025.

In the first series the coefficient of correlation between dry matter of feed consumed and heat production is somewhat higher than that in the second series. The latter was based on a considerably larger number (131) of measurements than the former (74) and is therefore considered as more significant.

There is a very close correlation between the dry matter of the feed consumed and carbon dioxide production, as is indicated by the high coefficient (0.938). The lowest coefficient of correlation was found between the dry matter of feed and the water vapor. This could be anticipated.

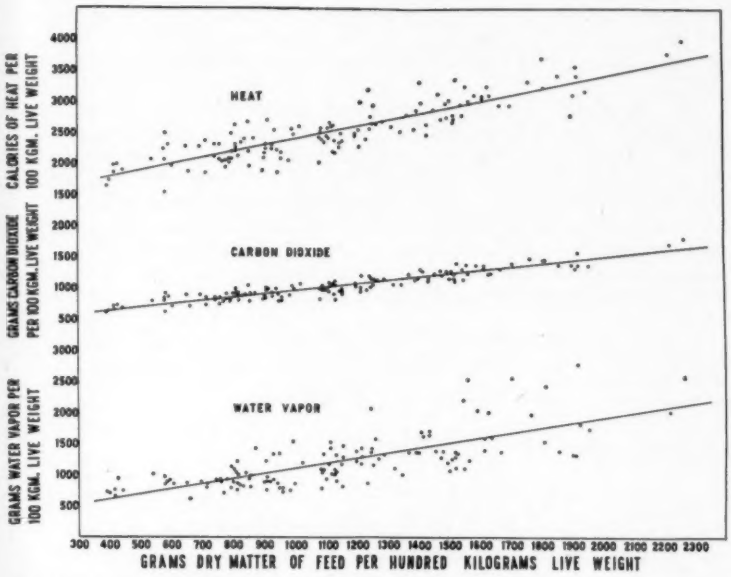
HEAT
CARBON DIOXIDE
WATER VAPOR
CALORIES OF HEAT PER 100 KGM. LIVE WEIGHT
GRAMS CARBON DIOXIDE PER 100 KGM. LIVE WEIGHT
GRAMS WATER VAPOR PER 100 KGM. LIVE WEIGHT
GRAMS DRY MATTER OF FEED PER HUNDRED KILOGRAMS LIVE WEIGHT

FIGURE 3.—Relationships (1) between dry matter of feed consumed and heat production, (2) between dry matter of feed consumed and carbon dioxide production, and (3) between dry matter of feed consumed and water vapor production of cattle

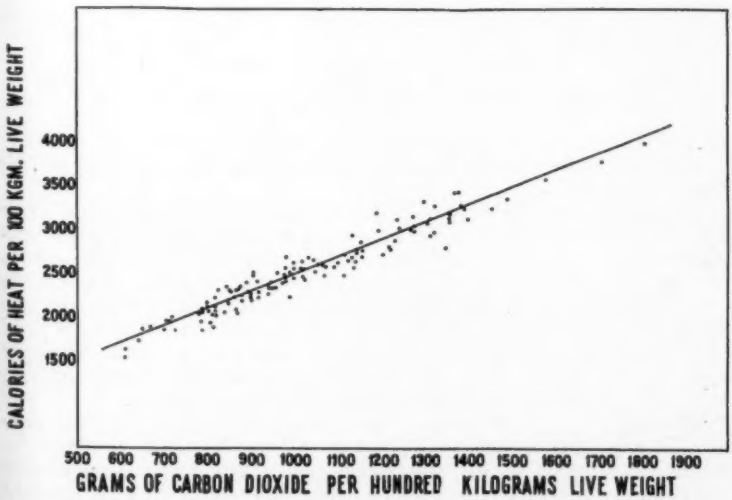

FIGURE 4.—Relationship between carbon-dioxide production and heat production of cattle

The correlation between the carbon dioxide production and the heat production is indicated graphically in Figure 4, in which calories of heat were plotted against grams of carbon dioxide. These data gave upon analysis the very high coefficient of correlation of 0.967 ± 0.004.

In all of the foregoing statistical considerations the data were computed to a uniform basis of live weight, namely, per 100 kgm. This was done to eliminate, or at least to minimize, the effect of the factor of live weight, since the measurements considered are functions of both the size of the animal and the rations consumed.

In Figure 5 the methane production per head was plotted against the dry matter of feed consumed per head. Since methane fermentation is a function of the feed, and not of the size of the animal, it was not deemed necessary in this case to take into account the live weights

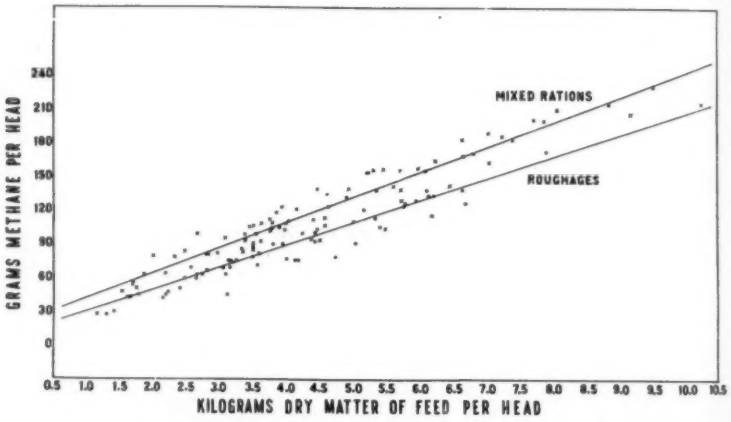

FIGURE 5.—Relationship between dry matter of feed consumed and methane production of cattle

of the animals. The coefficient of correlation between the methane production and the dry matter of feed, for all the experiments, was found to be 0.937 ± 0.007.

Of the 131 experiments of the second series 54 were on roughage and 77 were on rations consisting of roughage plus grain. In consideration of (1) the size of these two groups, (2) the greater content of digestible carbohydrates in rations containing grain, and (3) the known fact that methane fermentation is directly affected by the digestible carbohydrates of the rations, the data for dry matter and methane of the above two groups were analyzed separately, giving 0.942 ± 0.010 as the coefficient of correlation for the roughage rations and 0.963 ± 0.006 as the coefficient for the mixed rations.

The results of the statistical analysis of all the data under consideration are summarized in Tables 1 and 2. Table 1 presents the statistical constants, while the results of the final calculations, that is, the correlation coefficients and the prediction equations are presented in Table 2.

TABLE 1.—*Statistical constants for the daily measurements in the respiration calorimetric experiments*

Kind of measurement	Number of experiments	Range	Mean	Standard deviation	Coefficient of variation
Grams dry matter of feed per 100 kgm. live weight	74	302–2, 268	1, 208. 608± 36. 059	450. 862± 25. 476	38. 05±2. 40
Grams insensible loss per 100 kgm. live weight	74	584–3, 419	1, 668. 554± 43. 644	556. 603± 30. 836	33. 36±2. 05
Calories of heat per 100 kgm. live weight	74	1, 633–3, 995	2, 538. 446± 35. 519	452. 981± 25. 095	17. 84±1. 02
Grams dry matter of feed per 100 kgm. live weight	131	302–2, 268	1, 134. 237± 23. 825	404. 500± 16. 868	35. 66±1. 67
Calories of heat per 100 kgm. live weight	131	1, 531–3, 995	2, 528. 771± 27. 672	469. 814± 19. 591	17. 45± .75
Grams carbon dioxide per 100 kgm. live weight	131	609–1, 811	1, 028. 878± 13. 626	231. 346± 9. 647	22. 14± .97
Grams water vapor per 100 kgm. live weight	131	572–2, 803	1, 200. 221± 25. 498	432. 897± 18. 052	36. 07±1. 69
Grams dry matter of feed per head	131	1, 151–1, 022	4, 308. 557±109. 739	1, 863. 135± 77. 618	41. 08±1. 98
Grams methane per head	131	27– 230	106. 611± 2. 642	44. 859± 1. 871	42. 04±2. 04
Grams dry matter of feed per head	[a] 54	1, 300–7, 893	3, 946. 241±140. 662	1, 532. 381± 99. 452	38. 83±2. 88
Grams methane per head	[a] 54	27– 173	86. 833± 2. 949	32. 129± 2. 085	37. 00±2. 71
Grams dry matter of feed per head	[b] 77	1, 151–1, 022	4, 562. 649±155. 685	2, 025. 408±110. 182	44. 39±2. 85
Grams methane per head	[b] 77	27– 230	120. 481± 3. 632	47. 255± 2. 571	39. 22±2. 44

[a] Experiments on roughages only. [b] Experiments on mixed rations.

TABLE 2.—*Correlation coefficients of various pairs of measurements in the respiration calorimetric experiments, and prediction equations*

x—variable [c]	y—variable [c]	Number of experiments	Coefficient of correlation	Prediction equation
Dry matter of feed	Insensible loss	74	0. 818±0. 026	y=0. 9900x+ 472
Insensible loss	Heat production	74	.847± .022	y= .6892x+1, 389
Dry matter of feed	do	74	.936± .010	y= .9224x+1, 424
Do	do	131	.883± .013	y=1. 0250x+1, 366
Carbon dioxide	do	131	.967± .004	y=1. 9640x+ 508
Water vapor	do	131	.800± .021	
Dry matter of feed	Carbon dioxide	131	.938± .007	y= .5362x+ 421
Do	Water vapor	131	.762± .025	y= .8151x+ 276
Do	Methane	131	.937± .007	y= .0226x+ 9
Do	do	[b] 54	.942± .010	y= .0198x+ 9
Do	do	[a] 77	.963± .006	y= .0225x+ 18

[c] All values represented are per 100 kgm. live weight, except those for methane and the dry matter to which methane is related, which are per head.
[b] Experiments on roughages only.
[a] Experiments on mixed rations.

The results of Table 1 do not require extended comment; but in order that one may have a full appreciation of the significance of these data, especiallly in relation to variability, it is important to bear in mind the conditions represented by them.

In the first series, numbering 74 experiments, the data were derived from 58 experiments with steers, 8 experiments with lactating cows, and 8 experiments with dry cows. The animals varied in size from 311 to 642 kgm. live weight. The planes of nutrition varied from approximately one-half of the maintenance requirement to about three times maintenance. This extent of variation in plane of nutrition is indicated approximately by the range of variation in the

amount of dry matter of feed consumed. The rations can be characterized only very generally by the statement that in 17 experiments they consisted of roughages only, and in the remaining 57 experiments they consisted of a mixture of roughage and grain.

In the second series, comprising 131 experiments, the range of variation in feed consumption was the same as in the first series. Of the total number of experiments represented only 8 were with cows, and those were in dry condition. The remaining 123 were with steers. In some of these experiments the animals were quite young and of small size, while in others the subjects were mature. The average live weight varied from 162 to 655 kgm. In 54 experiments of this series the rations consisted of roughages only, while in 77 the rations were mixed.

In the light of the above-mentioned conditions the statistical constants presented in Table 1 are readily understandable, and are consistent.

The results exhibited in Table 2 are of theoretical as well as of practical significance.

It is of physiological interest to know how the heat production, the insensible loss, and the individual gaseous products, namely, the carbon dioxide, water vapor, and methane, are related to the feed consumption; as it is also of interest to know the degrees of interdependence of the heat production, the insensible loss, the water vapor, and the carbon dioxide. The interdependence of these measurements is indicated by the coefficients of correlation.

All the correlation coefficients shown in Table 2 are numerically high, and in comparison with their probable error are certainly to be regarded as significant. The highest correlation (0.967) is between the carbon dioxide production and the heat production. The correlation between the dry matter of feed and the insensible loss is the third lowest (0.818) of the series, that between the dry matter of the feed and water vapor being the lowest (0.762). The numerical values of these coefficients, as well as the data shown in Figures 1 and 3, indicate that the insensible loss and the water vapor—which constitutes the greater part of the insensible loss—are directly affected by the level of feeding; but the influence of other factors, such as have been discussed in the preceding paper (*8*), on the insensible loss, and presumably largely on the water vapor, are also apparent.

The prediction equations given in Table 2 are equations of a straight line, and represent the lines of regression shown in the several graphs previously referred to.

As regards the relation of the heat production to the dry matter of the feed, it has been demonstrated quite conclusively by Forbes, Braman, Kriss, et al. in recent investigations of this institute (*4*, *5*) that the heat production of cattle from fast to two or three times maintenance is not accurately represented by a straight line, but is represented by a curve of a higher order. Even between the planes of half maintenance and three times maintenance the heat production of an individual animal would not be accurately represented by a straight line, according to the evidence just cited. However, from a statistical point of view, the results on various feeds and numerous individual animals having been considered together, it appears justifiable although not quite in harmony with the established facts,

to represent the relation of the heat production to the dry matter of feed, within the range here considered, by the slope of a straight line, especially inasmuch as the linearity of regression of the heat production on the dry matter is evidenced by the graphical representation of the data (figs. 1 and 3), and by the high coefficients of correlation between the heat production and the dry matter of feed shown in Table 2.

APPLICATION OF PREDICTION EQUATIONS

The application of the prediction equations to the computation of the unknown variables is extremely simple, but it might be desirable to illustrate it by means of an example. The following is an example of the computation of the heat production (y—variable) from the carbon dioxide production (x—variable).

The carbon dioxide production of a steer weighing 486 kgm. per day was 5,950 gm. The daily production of carbon dioxide per 100 kgm. live weight is, therefore, 1,224 (5,950÷4.86) gm. This value is represented by x in the equation $y=1.964\ x+508$, in which y represents heat production per 100 kgm. live weight. Substituting 1,224 for x in the equation, we find $y=1.964$ times $1,224+508=2,912$ Cals. The daily heat production per head is, therefore, 14,152 (2,912 times 4.86) Cals.

In a similar manner the heat production of this steer may be computed from the dry matter of the feed consumed by substituting the number of grams of dry matter per 100 kgm. of live weight, which was 1,519, for x in the equation $y=1.025\ x+1,366$ and solving for y; and then multiplying the result by 4.86. The heat production thus computed would equal 14,206 Cals.

It is evident from the above example that it is essential to know the live weights of the animals in making use of the prediction equations in which x and y represent values per 100 kgm. of live weight. The computation of methane production from the dry matter of feed does not require a consideration of the live weights, since the x and y represent values per head. The heat production is expressed in Calories, and all the other measurements must be expressed in grams.

The computation of the heat production from the carbon dioxide production, as illustrated by the above example, differs fundamentally from the heat-CO_2 method of Armsby, Fries, and Braman (*2*) and of Braman (*3*). The principal difference lies in the fact that the computation of the heat production from the equation representing the direct relationship between heat production and CO_2 production, given in Table 2, and as used in the example cited, is direct and does not require any consideration of feed consumption. According to the heat-CO_2 ratio method, however, it is necessary first to compute the ratio of heat to CO_2 from an equation representing the relationship between heat-CO_2 ratios and air-dry matter of feed; and then the heat is computed by multiplying the ratio found by the known amount of CO_2. In view of the fact that the kind and composition of the feed must, of necessity, be disregarded in the determination of the heat-CO_2 ratio relating to the given feed, it would appear that the latter procedure makes the computation of the heat production from the CO_2 production unnecessarily complicated.

A comparison of the equations representing the relationship between the methane and the dry matter of roughage rations and mixed rations shows that the mixed rations give rise to a higher production of methane than do the roughages. This difference, amounting to 12 gm. of methane per kilogram of dry matter of feed, is indicated by the slight difference in the slope of the lines (fig. 5) represented by the equations and by the difference in the constant, and is attributable to the relatively larger amounts of digestible carbohydrates in the dry matter of the mixed rations than in the roughage rations.

An important question to consider in regard to the practical use of the data of Table 2 is the relative accuracy with which the heat production and the various gaseous products can be computed from the equations. The coefficients of correlation are a fair but not always a true index of such accuracy. For example, it would not be correct to assume that the methane production can be computed from the dry matter of feed with an accuracy of the same order as would characterize the computation of the carbon dioxide from the dry matter, on the strength of the fact merely that the coefficients of correlation are identical and are based upon the same number of experiments. The relative size of the means of the dependent variable seems to play an important rôle in this regard, in addition to the other two factors mentioned. Thus, one may expect to obtain a greater accuracy in the computation of the carbon dioxide production (which has a much greater mean numerical value than methane) from the dry matter of feed than in the computation of the methane production from the same. It would seem to be reasonable, however, to assume, on the basis of a comparison of correlation coefficients representing the same number of experiments, that the evaluation of the heat production from the carbon dioxide is more nearly accurate than is the evaluation of the same from the dry matter, and that the heat production can be computed from the dry matter of the feed with an accuracy of a higher order than that obtained in the computation of the same from the insensible loss.

A test of the relative accuracy with which the heat production can be computed by means of each of the three different prediction equations, and of the order of accuracy of the computation of the various gaseous products from the dry matter of the feed, is afforded by Table 3, in which a comparison is made of the computed values with the values which were experimentally determined, the data from several early and several recent experiments being used for this purpose.

The dry matter-heat equation used in this comparison was that based on the larger number of experiments, namely, $y=1.0250+1366$. The equation used for computing the methane was $y=0.0226\,x+9$.

The average percentage differences between the computed and the directly determined values in Table 3, disregarding the signs, are: Carbon dioxide computed from dry matter of feed, 4.7 per cent; methane computed from dry matter of feed, 10.6 per cent; water vapor computed from dry matter of feed, 14.9 per cent; heat production computed from insensible loss, 7.9 per cent; heat production computed from dry matter of feed, 4.9 per cent; heat production computed from carbon dioxide, 2.9 per cent.

TABLE 3.—*A comparison of directly determined values for heat production, carbon dioxide, methane, and water vapor with values computed from the prediction equations*

Experiment and animal No.	Period No.	Differences between actual and computed values for—					
		Water vapor	Methane	Carbon dioxide	Heat production from—		
					Insensible-loss equation	Dry-matter equation	Carbon-dioxide equation
Experiment 238:		*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Steer 47	1	+18.1	−3.9	+0.9	+4.7	+5.0	+4.6
36	2	+6.2	+3.1	−2.5	−5.0	−.5	+1.9
47	3	−8.7	−3.6	+.3	+9.5	+7.1	+6.9
36	4	−12.8	−5.8	−2.1	+2.8	+2.8	+4.5
47	5	−17.9	−17.6	+3.6	+28.6	+8.4	+4.4
36	6	−15.0	−16.8	−1.6	+22.4	+2.6	+2.1
47	7	+1.5	−13.2	+4.3	+15.8	+12.0	+8.0
36	8	+2.1	−15.3	+.2	+8.5	+6.0	+5.4
47	9	−4.3	+10.7	+15.7		+13.1	+.9
36	10	+4.0	+12.3	+9.6	−.6	+9.5	+1.8
Experiment 237:							
Steer 47	2	−21.6	−9.2	−6.7	−7.9	−2.2	+3.4
254	3	−21.8	−31.4	−8.6	+6.2	−4.8	+1.1
47	4	−21.2	−14.0	+.1	+9.7	+1.4	+1.0
254	5	−31.4	−.3	−3.0	+8.8	−6.2	−4.2
47	6	−17.1	−4.0	+7.9	+10.3	+4.7	−1.5
254	8	−20.0	−8.0	−6.6	−7.8	−6.4	−1.2
36	10	−11.0	+29.6	+7.5	−3.1	+2.3	−3.3
47	11	−5.2	+18.5	+7.0	−.3	+3.3	−2.1
36	12	+1.1	−9.1	+1.7	−1.7	+2.4	+1.0
47	13	+6.6	−8.6	+2.2	−3.7	+2.8	+1.0
Experiment 235:							
Steer 259	2	+27.7	+12.4	−2.5	−7.7	−2.9	−1.1
260	2	−21.2	+3.8	+3.5	+16.7	+5.7	+2.6
259	3	+19.9	+.2	−3.7	−12.6	−3.7	−.3
260	3	−14.5	+8.0	+4.3	+10.8	+7.4	+4.0
Experiment 211:							
Steer D	1	+36.5	+12.2	+9.1	−7.6	+9.5	+2.3
D	2	+9.3	−16.7	−4.9	−3.0	−1.0	+2.5
D	3	+20.8	−6.7	−.4	+10.1	+3.9	+4.8
D	4	+27.3	+1.2	+7.0		−4.7	+.8
D	5	−4.5	+7.3	−1.0	−3.6	−3.5	−3.8
G	1	+25.8	+7.1	+1.3		−1.3	−2.0
G	2	−5.2	−20.9	−14.0	−2.4	−1.2	−1.1
G	3	−23.2	−9.8	−8.4	−2.9	+5.1	+3.0
G	4	−6.4	+6.9	−1.2	−3.5	−4.7	−4.2
G	5	−14.5	+5.4	−1.8	+3.5	−3.8	−3.5
Experiment 221 F:							
Cow 874	1	+3.5	−8.8	+2.7	+7.1	+9.3	+7.3
874	2	+35.4	−7.9	+10.6	−1.4	+7.8	−.5
887	1	+.6	−16.1	−5.2	−13.7	−1.9	+3.0
887	2	+11.8	−15.8	−6.6	−14.2	−3.6	+1.8
Average		14.9	10.6	4.7	7.9	4.9	2.9

The data of Table 3 show, therefore, in terms of percentage that the computation of the carbon dioxide from the dry matter of the feed is relatively more accurate than is the computation of the methane from the same; that the computation of the water vapor is the least accurate; that the heat production can be computed from the carbon dioxide with a close approximation to the truth; and that the dry matter of the feed is a better basis for predicting the heat production than is the insensible loss.

Obviously, if the signs of the individual differences between the actual and the computed values in Table 3 were taken into account, the average differences would be considerably smaller than those indicated above.

It should be realized, however, that the experimental data upon which the results of Table 3 are based constitute part of the data from which the prediction equations under consideration were deduced, and that the results of Table 3 do not indicate the maximum deviations from the truth possible on application of these equations to other data.

It seems safe to assume that these prediction equations would apply more nearly correctly to large groups of animals (a barnful, for example) than to single individuals, and that these equations do not provide means for the computation of the heat production and of the various gaseous products of an animal with a sufficient degree of accuracy for the most critical purposes.

SUMMARY

This paper reports numerical and graphic results of a biometric study of relations between values representing the feed consumption, the production of heat, carbon dioxide, water vapor, and methane, and the insensible loss in body weight of cattle, derived from published respiration calorimetric experiments of the Institute of Animal Nutrition of the Pennsylvania State College.

Two series of data were subjected to statistical analysis. Those of the first series were derived from 74 experimental periods (58 with steers, and 16 with cows), and included data for insensible loss, heat production, and dry matter of feed, these experiments representing planes of nutrition varying between about one-half maintenance and about three times the maintenance energy requirement. The second series comprised the results of 131 experimental periods (123 with steers and 8 with cows), including data for heat, the production of carbon dioxide, methane, and water vapor, and for the dry matter of the feed, of rations representing planes of nutrition varying as in the first series.

In the first series the insensible loss and the heat production were each correlated with the dry matter of the feed, and the insensible loss was correlated with the heat production. In the second series the heat production, the carbon dioxide, the water vapor, and the methane were each correlated with the dry matter of the feed, and the carbon dioxide and the water vapor were each correlated with the heat production. The correlation between the methane and the dry matter of the feed was also determined separately for two groups of data of the second series, one representing roughage rations, the other representing mixed rations consisting of roughage and grain. The following correlation coefficients were obtained: Between dry matter of feed and insensible loss, 0.818 ± 0.026; between dry matter of feed and carbon dioxide, 0.938 ± 0.007; between dry matter of feed (all rations) and methane, 0.937 ± 0.007; between dry matter of feed (roughages) and methane, 0.942 ± 0.010; between dry matter of feed (mixed rations) and methane, 0.963 ± 0.006; between dry matter of feed and water vapor, 0.762 ± 0.025; between dry matter of feed and heat production (74 periods), 0.936 ± 0.010; between dry matter of feed and heat production (131 periods), 0.883 ± 0.013; between carbon dioxide and heat production, 0.967 ± 0.004; between insensible loss and heat production, 0.847 ± 0.022; between water vapor and heat production, 0.800 ± 0.021.

Regression equations were worked out from the data by means of which the heat production, the carbon dioxide, the water vapor, and the methane production can be computed, with various degrees of accuracy, from the dry matter of the feed consumed; and similar equations were worked out by means of which the heat production can be computed from either the carbon dioxide production, the insensible loss, or the dry matter of the feed.

A comparison of the computed values (by the use of the regression equations) with the values which were directly determined in a number of experiments of this institute, gave the following average, percentage differences, when the signs of the individual differences were disregarded: Carbon dioxide computed from dry matter of feed, 4.7 per cent; methane computed from dry matter of feed, 10.6 per cent; water vapor computed from dry matter of feed, 14.9 per cent; heat production computed from insensible loss, 7.9 per cent; heat production computed from dry matter of feed, 4.9 per cent; heat production computed from carbon dioxide, 2.9 per cent.

The conclusion is drawn that for practical purposes the dry matter of the feed consumed is a better basis for predicting the heat production of cattle than would be the insensible loss in body weight.

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